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(71) Applicant (for all designated States except US):  
**PALATIN TECHNOLOGIES, INC.** [US/US]; 103  
Carnegie Center, Suite 200, Princeton, NJ 08540 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SHARMA, Shubh,**  
**D.** [US/US]; 6 Petty Road, Cranbury, NJ 08512 (US). **SHI,**  
**Yi-Qun** [CN/US]; 18 Aldrich Street, East Brunswick, NJ  
08816 (US).

(74) Agent: **SLUSHER, Stephen, A.;** Peacock, Myers  
& Adams, P.C., P.O. Box 26927, Albuquerque, NM  
87125-6927 (US).

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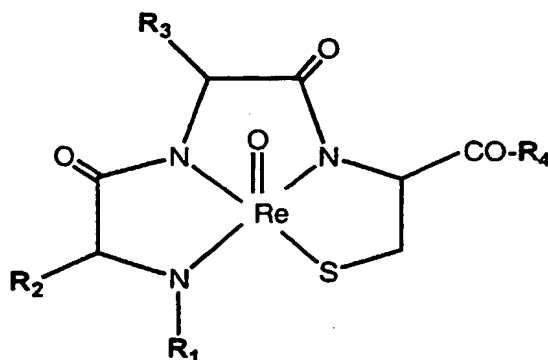
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(54) Title: IDENTIFICATION OF TARGET-SPECIFIC FOLDING SITES IN PEPTIDES AND PROTEINS



(I)

(57) Abstract: The invention provides methods for identification and determination of target-specific folding sites in peptides and proteins, including a method for determining a secondary structure binding to a target of interest within a known parent polypeptide that binds to the target of interest. In one embodiment of the invention, a residue or mimetic containing a nitrogen atom and a sulfur atom available for binding to a metal ion is serially substituted for single residues in or inserted between two adjacent residues in a known primary sequence of a peptide or protein. The resulting sequence, which includes a minimum of the residue or mimetic containing a nitrogen atom and a sulfur atom available for binding to a metal

ion and two residues on the amino terminus side thereof, is complexed with a metal ion, thereby forming a metallopeptide. The resulting metallopeptides are then used in binding or functional assays related to the target of interest, and the metallopeptide demonstrating binding or functional activity is selected. The invention further provides methods to determine the specific sequence and local three-dimensional structure of that portion of peptides or proteins that bind to a receptor or target of interest, or mediate a biological activity of interest and methods to determine the pharmacophore of receptors or targets of interest. The invention provides for defined pharmacophores or receptors or targets of interest and directed libraries for identification and determination of target-specific folding sites in peptides and proteins and for identification and determination of pharmacophores of receptors or targets of interest.

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## PATENT APPLICATION

## IDENTIFICATION OF TARGET-SPECIFIC FOLDING SITES IN PEPTIDES AND PROTEINS

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CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the filing of U.S. Provisional Patent Application Serial No. 60/256,842, entitled *Iterative Deconvolution Of Target-Specific Folding Sites In Peptides*, filed on December 19, 2000; of U.S. Provisional Patent Application Serial No. 60/304,835, entitled

10 *Metallopeptides for Treatment of Alzheimer's and Prion Disease*, filed on February 13, 2001; and of U.S. Provisional Patent Application Serial No. 60/327,835, entitled *Urokinase-Type Plasminogen Activator Receptor Specific Metallopeptides*, filed on October 4, 2001; and the specification of each of the foregoing is incorporated herein by reference.

15

BACKGROUND OF THE INVENTIONField of the Invention (Technical Field):

The present invention relates to methods for identification and determination of target-specific folding sites in peptides and proteins; methods to determine the specific sequence and local three-dimensional structure of that portion of peptides or proteins that bind to a receptor or target of

20 interest, or mediate a biological activity of interest; methods to determine the pharmacophore of receptors or targets of interest; and directed libraries for identification and determination of target-specific folding sites in peptides and proteins and for identification and determination of pharmacophores of receptors or targets of interest.

25 Background Art:

Note that the following discussion refers to a number of publications by author(s) and year of publication, and that due to recent publication dates certain publications are not to be considered as prior art vis-a-vis the present invention. Discussion of such publications herein is given for more complete background and is not to be construed as an admission that such publications are prior art

30 for patentability determination purposes.

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*Peptide and Protein Folding.* Determination of the biologically relevant structure of proteins and peptides, which can be characterized as a functional three-dimensional structure, is a difficult problem in the biological, biochemical and pharmaceutical sciences. Through use of any of a variety of methods the primary structure of relevant peptides or proteins may be ascertained. That is, the sequence of amino acid residues composing the peptide or protein is known, and it is known that the peptide or protein has a desired biological effect, such as binding a target molecule or receptor of interest, mediating a biological activity of interest, or the like. However, both the three-dimensional structure and sequence of the portion of the peptide or protein forming a ligand and thereby giving rise to the desired biological effect is unknown.

Peptides and proteins are highly flexible, due in large part to amino group and carboxyl group bonds of individual amino acid residues having a high rotational degree of freedom. In addition, some bonds in side chains of individual amino acid residues also have rotational degrees of freedom. The non-bonded steric interactions between amino acid residues force the peptide or protein along its degrees of freedom into some stable minimal free energy configuration. Local structures, also known as the "secondary structure," are common in peptides and proteins. These structures include  $\alpha$ -helixes,  $\beta$ -bends, sheets, extended chains, loops and the like, and most often contribute to binding or receptor-specificity of peptides and proteins.

There are several types of  $\alpha$ -helixes known, differing in torsion angles within the amino acid residues of the actual turn and by the patterns of intra- and inter-molecular hydrogen bonding. There are also a number of known different  $\beta$ -bends, differing in the dihedral torsion angles  $\Psi$  (for the  $C^\alpha$ -C bond) or  $\Phi$  (for the  $C^\alpha$ -N bond), or both.

A wide variety of mathematical, computational and others models have been developed for predicting the secondary structure of proteins and the secondary and tertiary structure of peptides, but no model gives satisfactory responses under other than the most limited circumstances. For example, software modeling programs (e.g., such as those distributed by Tripos, Inc., PharmacoPeia Inc. and the like), depend on various algorithms, statistical tools, assumed relationships between groups and the like, any or all of which may not be valid for any given protein or peptide. A number of methods are described in the art, such as those disclosed in International Publication No. WO 00/23564 to Xencor, Inc., International Publication Nos. WO 00/57309 and WO 01/35316, both to Structural Bioinformatics, Inc., International Publication No. WO 01/50355 to Structural Bioinformatics

Advanced Technologies A/S, International Publication No. WO 01/59066 to Xencor, Inc., U.S. Patent No. 6,278,794 to Parekh et al., and U.S. Patent Application No. 2001/0000807 to Freire and Luque.

Generation of structure-based pharmacophores, utilizing experimental methods such as X-ray crystallography or NMR, optionally in conjunction with protein structure determination methods, such as homology modeling, is known in the art. However, in order for this approach to be employed it must be possible to obtain appropriate data from the ligand in the conformation specific for the receptor defining the pharmacophore. In many, if not most, instances this is not feasible.

It may be determined that a particular peptide or protein sequence, with a length between about five residues to about fifty or more residues, binds to a particular receptor. However, the specific residues actually participating in binding, and the local secondary structure of the sequence which contains these specific residues, is not known. Without this knowledge, it is impossible to devise a systematic rational approach to make peptide-based drugs, peptidomimetic drugs or other small molecule drugs. With knowledge of the specific residues and local secondary structure, it is possible to define the pharmacophore for the receptor. This definition may include, for example, the location in a three-dimensional construct of hydrogen bond donors and acceptors, positively and negatively charged centers, aromatic ring centers, hydrophobic centers and the like, preferably described in terms of the distances between the atoms in the pharmacophore.

U.S. Patent No. 5,834,250, to Wells et al., provides methods for the systematic analysis of the structure and function of polypeptides, specifically by identifying active domains by substituting a "scanning amino acid" for one of the amino acid residues within a suspected active domain of the parent polypeptide. These residue-substituted polypeptides are then assayed using a "target substance". In practice, a "scanning amino acid", such as alanine, is substituted for various residues in a polypeptide, and binding of the substituted polypeptide to a target substance compared to binding of the parent polypeptide. However, this method provides no direct information concerning the secondary structure of the active domain, nor information concerning the pharmacophore of the target substance. Similarly, U.S. Patent No. 6,084,066, to Evans and Kini, discloses homologues and analogs of naturally occurring polypeptides with "conformation-constraining moieties" flanking "interaction sites". However, this method requires that the "interaction site" or amino acid sequence be known. The "interaction site" sequence is then flanked on both termini with proline residues, which are asserted to stabilize interaction sites. This method similarly provides no direct information

concerning the secondary structure of the "interaction site", nor information concerning the pharmacophore of the target substance.

There is thus a significant and substantial need to develop methods for identifying the specific residues in a peptide which are involved in binding to a receptor of interest, and to identify the

5 specific secondary structure of the residues involved in binding.

*Metallopeptides.* It is known that linear peptides have high rotational degrees of freedom, such that for even small peptides with known primary structures the theoretically possible secondary and tertiary structures may number in the millions. In general cyclic peptides are more constrained, and at least small cyclic peptides have far fewer theoretically possible secondary and tertiary

10 structures. However, even with cyclic peptides it is frequently impossible to predict with precision the actual secondary structures present in such peptide. By contrast, metallopeptides have well-defined and limited secondary structures, with the residues involved in metal ion complexation forming a turn structure about the metal ion. The atoms forming a part of the coordination sphere of the metal ion are fixed by the coordination geometry of the metal ion. This, coupled with the peptide bonds

15 between residues and the side chain bonds, yields a conformationally fixed and predictable secondary structure for at least the residues involved in metal ion complexation. U.S. Patent No. 5,891,418, entitled *Peptide-Metal Ion Pharmaceutical Constructs and Applications*, U.S. Patent No. 6,027,711, entitled *Structurally Determined Metallo-Constructs and Applications*, and P.C.T. Patent Application Serial No. PCT/US99/29743, entitled *Metallopeptide Combinatorial Libraries and*

20 *Applications*, each teach aspects of making and using metallopeptides and mimetics thereof, and each of the foregoing is incorporated herein by reference. These patents and applications disclose receptor-specific metallopeptides and methods of making peptides and complexing the peptides to various metal ions.

There are methods for screening peptides for metal coordinating properties, such as

25 disclosed in U.S. Patent No. 6,083,758 to Imperiali and Walkup. However, these methods, which employ monitoring the fluorescence to detect metal coordination, do not provide any information regarding binding of metal coordinated peptides to receptors or targets of interest.

SUMMARY OF THE INVENTION (DISCLOSURE OF THE INVENTION)

In accordance with one aspect of the present invention there is provided a method of determining a secondary structure binding to a target of interest within a known parent polypeptide that binds to the target of interest. The parent polypeptide may be a peptide, a polypeptide or a protein. Such method includes (a) providing a known parent polypeptide that binds to a target of interest with a known primary structure, such primary structure consisting of  $n$  residues; (b) constructing a first peptide of the formula  $R_1$ -C- $R_2$ ; (c) complexing the first peptide of the formula  $R_1$ -C- $R_2$  to a metal ion, thereby forming a first  $R_1$ -C- $R_2$  metallopeptide; (d) screening the first  $R_1$ -C- $R_2$  metallopeptide for binding to the target of interest; (e) repeating steps (b) through (d) as required, wherein the resulting  $R_1$ -C- $R_2$  metallopeptide differs in at least either  $R_1$  or  $R_2$ ; and (f) selecting the  $R_1$ -C- $R_2$  metallopeptide exhibiting binding to the target of interest, whereby such  $R_1$ -C- $R_2$  metallopeptide comprises the secondary structure binding to the target of interest. In the formula  $R_1$ -C- $R_2$ ,  $R_1$  includes from 2 to  $n$  residues, which residues are the same as or homologues of residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure. C is a residue or mimetic thereof providing both an N and an S for metal ion complexation.  $R_2$  includes from 0 to  $n-2$  residues, which residues are the same as or homologues of residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure.  $R_1$  and  $R_2$  together form a sequence in the same order as in the parent polypeptide primary structure with C either inserted between two adjacent residues corresponding to two adjacent residues in the primary structure or substituting for a single residue corresponding to a single residue in the primary structure. C may be an L- or D-3-mercapto amino acid, including L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homologue of any of the foregoing. In a preferred embodiment,  $n$  is at least 3.

The number of residues included in  $R_1$  and  $R_2$  together can be less than  $n$ , equal to  $n$  or greater than  $n$ . For polypeptides where  $n$  is at least 15, the method can further include the step of dividing the primary structure into at least three divided primary structures, each such divided primary structure overlapping the primary structure of each adjacent divided primary structure by at least two residues, and thereafter following steps (b) through (f) with respect to each such divided primary structure. The peptides of the formula  $R_1$ -C- $R_2$  can include an N-terminus free amino group or acetyl group and can independently include a C-terminus free carboxylate or amide group.

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The metal ion in this and in other methods and constructs of this invention may be an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or Gd. The resulting  $R_1$ -C- $R_2$  metallopeptides, and other metallopeptides of this invention, are stable in solution. For some metal ions, the  $R_1$ -C- $R_2$  metallopeptides, and other metallopeptides of this invention, form a stable solid when not in solution. Re is a particularly preferred ion, and forms a stable solid metallopeptide when not in solution.

The target of interest in this method and other methods of this invention may be a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

Any method of screening for binding to the target of interest may be employed. In one embodiment, the method of screening for binding includes competing a known binding partner for binding to the target of interest with the  $R_1$ -C- $R_2$  metallopeptide, such as in a competitive inhibition assay. In such an assay, the parent polypeptide may be utilized as the known binding partner. Alternatively, a peptide derived from the parent polypeptide, which derived peptide binds to the target of interest, may be employed. The method of screening for binding to the target of interest may also include a functional assay. In one embodiment, employed where the target of interest is a biological receptor capable of transmitting a signal, the method of screening includes determining whether the  $R_1$ -C- $R_2$  metallopeptide induces transmission of the signal, and is thus an agonist. In a related embodiment, the method of screening includes determining whether the  $R_1$ -C- $R_2$  metallopeptide inhibits transmission of the signal in the presence of a binding partner to the target of interest known to induce transmission of the signal, and is thus an antagonist.

In this method,  $R_1$  and  $R_2$  can each contain residues that are the same as residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure. In an alternative embodiment, one or more residues are substituted with homologues. Thus any cysteine residue in  $R_1$  or  $R_2$  can be substituted with a homologue that does not contain a free sulfhydryl group. Suitable homologues that can be substituted for a cysteine include glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residues. Alternatively, the cysteine can be substituted with an S-protected cysteine, such that the sulfur atom in the cysteine cannot form a complex with the metal ion. In general, the cysteine can be substituted with a neutral mimetic of an amino acid residue of less than about 150 MW. Any proline residue in the two residues immediately

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adjacent the amino-terminus side of C are preferably substituted, and may be substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue. In general, any proline residue can be substituted with a neutral mimetic of an amino acid of less than about 150 MW that provides an N for metal ion complexation.

- 5           The peptides of the formula  $R_1-C-R_2$  are preferably constructed by a chemical method of peptide synthesis. Such methods include solid phase synthesis and solution phase synthesis. In one advantageous embodiment, C can include an orthogonal S-protecting group compatible with the chemical method of peptide synthesis, which orthogonal S-protecting group is characterized by being cleavable at or prior to metal ion complexation. In yet another embodiment the peptides of the
- 10          formula  $R_1-C-R_2$  are constructed by expression in biological systems, in which embodiment the method can include use of a recombinant vector.

- In accordance with another aspect of the present invention there is provided a related but different method of determining a secondary structure binding to a target of interest within a known parent polypeptide that binds to the target of interest. In this method, a parent polypeptide with a
- 15          known primary structure that binds to a target of interest comprising n amino acid residues is provided, wherein n is at least 3. At least one construct is then made, the construct including at least three elements. One element is an  $N_1S_1$  element with an  $\alpha$ -amino group that provides both an N and an S for complexation to a metal ion. The remaining at least two elements each include an  $\alpha$ -amino group and an  $\alpha$ -carboxyl group and provide an N for complexation to a metal ion. These at least two
- 20          elements are the same as or homologous with and in the same order as residues in the parent polypeptide with a known primary structure. The at least three elements are joined by peptide bonds and ordered such that the  $N_1S_1$  element is on the carboxyl terminus end of the at least two elements, thereby forming an  $N_1S_1$  element-containing construct. The resulting  $N_1S_1$  element-containing construct is then complexed to a metal ion, thereby forming a metalloconstruct. The metalloconstruct
- 25          is screened for binding to the target of interest. The foregoing steps are repeated as required, in each instance with the remaining at least two elements including at least one different residue in the parent polypeptide with a known primary structure. The metalloconstruct exhibiting the highest binding to the target of interest is then selected. In this method, the  $N_1S_1$  element can optionally be the carboxyl terminal end element of the construct. In one embodiment, the  $N_1S_1$  element-containing
- 30          construct includes at least four elements, the four elements consisting of an  $N_1S_1$  element with an  $\alpha$ -



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amino group and the remaining at least three elements including an  $\alpha$ -amino group and an  $\alpha$ -carboxyl group, the remaining at least three elements being the same as or homologous with and in the same order as residues in the parent polypeptide with a known primary structure. In this embodiment, the N<sub>1</sub>S<sub>1</sub> element is on the carboxyl terminus end of any two of the at least three elements, and the at least four elements are joined by peptide bonds. In yet another embodiment of this method, the at least two elements are amino acid residues, and optionally such amino acid residues are alanine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, methionine, proline, glutamine, arginine, serine, threonine, valine, tryptophan or tyrosine. The amino acid residues may be L-amino acid residues, D-amino acid residues, a combination of L-amino acid residues and D-amino acid residues, or any modified protein amino acid residues, non-protein amino acid residues, mimetics of non-protein amino acid residues, mimetics of protein amino acid residues, post-translationally modified amino acid residues, or enzymatically modified amino acid residues. The number of elements in a construct of this method may be less than n, equal to n, equal to n + 1 or greater.

In accordance with another aspect of this invention there is provided a method of determining a metallopeptide that binds to a target of interest. In this method a known amino acid sequence with a known primary structure of n residues, where n is at least 3, is selected, which known amino acid sequence binds to the target of interest. A library of amino acid sequences is then designed by selecting at least two consecutive residues from a stretch of consecutive residues in the known primary structure and inserting a residue providing both an N and S for metal ion complexation on the carboxy terminal end of two of the at least two selected consecutive residues. Each such designed sequence constitutes a library member. Each library member differs by at least one residue or the location of the insertion of the residue providing both an N and S for metal ion complexation. The library of designed amino acid sequences is then constructed, using any method of peptide synthesis, and each library member of designed amino acid sequences is complexed to a metal ion, thereby forming a library of metallopeptides. Each member of the library of metallopeptides is then screened for binding to the target of interest, and a metallopeptide exhibiting binding to the target of interest is selected. In a related embodiment, at least one residue of the selected at least two consecutive residues is a homologue of the corresponding residue in the stretch of consecutive residues in the known primary structure.

In accordance with another aspect of this invention another method of determining a metallopeptide that binds to a target of interest is provided. In this method a known amino acid sequence with a known primary structure of  $n$  residues, where  $n$  is at least 4, is selected, which known amino acid sequence binds to the target of interest. A library of amino acid sequences is then  
5 designed by selecting at least three consecutive residues from a stretch of consecutive residues in the known primary structure and substituting a residue providing both an N and S for metal ion complexation for the carboxy terminal residue of any consecutive stretch of three of the at least three selected consecutive residues. If the selected at least three consecutive residues are more than three residues, then the residue providing both an N and S for metal ion complexation need not be  
10 the carboxy terminal residue of resulting amino acid sequence, so long as the residue is substituted for the carboxy terminal residue in any group of three consecutive residues. Each sequence constitutes a library member, the library members being characterized in that each differs by at least one residue from any other library member. The library is then constructed and the library members complexed to a metal ion, thereby forming a library of metallopeptides. Each member of the library is  
15 then screened for binding to the target of interest and a metallopeptide exhibiting binding to the target of interest is selected. In a related embodiment, at least one residue of the selected at least two consecutive residues is a homologue of the corresponding residue in the stretch of consecutive residues in the known primary structure.

In accordance with yet another embodiment of this invention a method of determining a  
20 target-specific binding pharmacophore for a target of interest is provided. In this method, a metallopeptide binding to a target of interest is selected by means of any of the forgoing methods. Utilizing the selected metallopeptide, the spatial position of amino acid side chains in and immediately adjacent the metal ion coordination site is determined by building a molecular model based on the coordination geometry of the metal ion. This thus defines a target-specific binding  
25 pharmacophore for the target of interest. This method can optionally further include optimizing binding of the selected metallopeptide to the target of interest by changing the chirality of one or more of the amino acid residues complexed to the metal ion, or amino acid residues adjacent to the amino acid residues complexed to the metal ion. This method can also optionally further include  
30 optimizing binding of the selected metallopeptide to the target of interest by substituting a natural or synthetic homologue for at least one amino acid residue complexed to the metal ion, or at least one

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amino acid residue adjacent to the amino acid residues complexed to the metal ion. After such optimization, if the optimized metallopeptide provides improved binding to the target of interest, then the optimized metallopeptide is utilized for building a molecular model based on the coordination geometry of the metal ion. In this method, computer-based modeling can be employed to build the molecular model. This molecular model, and hence the pharmacophore, may include the location in a three-dimensional model of hydrogen bond donors and acceptors, positively and negatively charged centers, aromatic ring centers, hydrophobic centers and the like. In a preferred embodiment, the resulting pharmacophore is described in terms of spatial location of atoms in the pharmacophore and the distances between the atoms in the pharmacophore

In accordance with yet another embodiment of this invention a target-specific binding pharmacophore for a target of interest is provided. The pharmacophore is defined by a metallopeptide, selected such that the metallopeptide binds to the target of interest. The metallopeptide includes a residue providing both an N and an S for metal ion complexation and, joined by a peptide bond to the amino-terminus side of such residue, at least two consecutive residues that are the same as or homologues of the same number of consecutive residues of the primary structure of a known sequence of amino acid residues that binds to the target of interest. A metal ion is complexed to the residues. Any proline residue in the two residues immediately adjacent the amino-terminus side of the residue providing both an N and an S for metal ion complexation is substituted with a residue providing an N for metal ion complexation. Any residue with a free sulfhydryl group, other than the residue providing both an N and an S for metal ion complexation, is substituted with a homologue not containing a free sulfhydryl group. The pharmacophore thus provided may be further defined by the spatial position of amino acid side chains in and immediately adjacent the metal ion coordination site, such as by use of a molecular model based on the coordination geometry of the metal ion.

In accordance with yet another embodiment of this invention a library of metallopeptides targeted to a target of interest is provided. Each constituent library member includes an amino acid sequence of the formula  $R_1-C-R_2$ , defined as set forth above, with a metal ion complexed to each library member. Representative libraries, as set forth in the examples contained herein, include libraries of metallopeptides targeted to the urokinase-type plasminogen activator receptor, melanocortin receptors, vasopressin receptor, oxytocin receptor or angiotensin receptor, or libraries

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constituting amyloid beta-protein related peptides for treatment of Alzheimer's disease or peptides for treatment of prion disease.

It is a primary object of this invention to provide conformationally-constrained metallopeptides as surrogates for naturally-occurring structural motifs, such as those motifs commonly found in naturally-occurring peptides and proteins, including reverse turn structures, type I, II and III beta turns, gamma turns, inverse gamma turns, and short helical, sheet and extended chain structures. A secondary structural motif is necessarily defined by a conformationally-constrained metallopeptide, which secondary structural motif mimics, or can be made to mimic, the topologies found in naturally occurring structural motifs. The secondary structural motif formed as a consequence of metal ion complexation in the metallopeptide is more stable than the naturally occurring secondary structural motifs, which are generally stabilized only by weaker interactions such as van der Waals' interactions and hydrogen bonds.

Another object of this invention is to provide backbone structures of turns formed upon complexation of a metal ion to an amino acid sequence including an  $N_1S_1$  residue, forming a secondary structural motif with substantial topological similarities to classical protein turn structures. Amino acid side chains associated with the metal ion-induced turn can be topographically positioned such that they occupy the same chemical space as the corresponding side chains in classical turn structures.

Another object of this invention is to provide libraries of metallopeptides based upon a known amino acid sequence that exhibits binding to a target or receptor of interest, wherein the peptides include a metal ion-complexing domain, such that a specific conformational structure providing a secondary structural motif is obtained upon metal complexation.

Another object of this invention is to provide metallopeptide sequences, wherein the metallopeptides include a metal ion-complexing domain, such that a specific conformational secondary structural motif is obtained upon metal complexation.

Another object of this invention is to provide metallopeptide sequences, wherein the metallopeptides include a metal ion-complexing domain in a distinct and known location within the sequence, wherein the metallopeptides may be exposed to a substance and one or more metallopeptides will exhibit specificity and affinity for the substance.

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Another object of this invention is to provide a method for identifying the specific residues within a known peptide that are involved in binding to a known target of interest.

Another object of this invention is to provide methods for synthesis of peptides wherein the peptides contain a single reactive -SH group forming a part of a metal ion-complexing domain, whereby the reactive -SH group is protected during synthesis, and is deprotected only upon  
5 complexing the peptide with a metal ion.

Another object of this invention is to provide a method for making metallopeptides as models for the active binding site in a known parent polypeptide, wherein each endogenous cysteine residue is substituted or, alternatively, wherein each endogenous cysteine residue further includes an S-  
10 protecting group, such that the sulfur of such endogenous cysteine does not form a part of a metal ion-complexing domain.

Another object of this invention is to provide libraries of peptides wherein each of the peptides forming the library contains a secondary structural motif upon complexation with metal ion, thereby forming a metallopeptide.

Another object of this invention is to provide libraries containing metallopeptides with high  
15 specificity and affinity for a target molecule of interest, such high specificity and affinity resulting from each of the metallopeptides forming the library containing a secondary structural motif as a consequence of metal ion complexation.

Another object of this invention is to provide a method for rapid and efficient complexation of a  
20 pool of diverse peptides with a metal ion, including a rhenium metal ion.

Other objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The objects and  
25 advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and form a part of the specification,  
30 illustrate one or more embodiments of the present invention and, together with the description, serve

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to explain the principles of the invention. The drawings are only for the purpose of illustrating one or more preferred embodiments of the invention and are not to be construed as limiting the invention.

In the drawings:

**Fig. 1 A** is backbone diagram of the peptide sequence Ala-Ala-Ala-Cys-Ala (SEQ ID NO:1) complexed to a rhenium metal ion, with the Ala-Ala-Cys sequence forming the metal ion binding domain, thereby forming a metallopeptide;

**Fig. 1 B** is a backbone peptide coordinate diagram of a classical beta-II' turn;

**Fig. 1 C** is the diagram of **Fig. 1 A** superimposed on the diagram of **Fig. 1 B**, aligned at the respective C- $\alpha$  carbon atoms of the three consecutive N-terminal amino acid residues. Comparison of the superimposed structures demonstrates excellent overlap at the three C- $\alpha$  carbon atom positions, with a calculated root mean square deviation (RMSD) per atom of  $< 0.05 \text{ \AA}$ . The metal ion located in the center of the turn of the diagram of **Fig. 1 A** corresponds to the hydrogen bond that stabilizes the natural beta turn structure of **Fig. 1 C**. In this representation, three C- $\beta$  carbon atoms of the metallopeptide are pointed in directions other than those in natural beta-turn structure, thereby providing access to additional chemical space. The C-terminal end of the metallopeptide further provides access to new chemical space;

**Fig. 2 A** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion, with the Ala-Ala-D-Cys sequence forming the metal ion binding domain, thereby forming a metallopeptide;

**Fig. 2 B** is the diagram of **Fig. 2 A** superimposed on the diagram of **Fig. 1 B** in a manner similar to that depicted in **Fig. 1 C**. A comparison of these structures reveals excellent overlap at three C- $\alpha$  carbon atoms of the three consecutive N-terminal amino acid residues, similarly with an overlap of RMSD  $< 0.05 \text{ \AA}$ . The metal ion located in the center of the turn similarly corresponds to the hydrogen bond that stabilizes the natural beta turn structure. In this representation, C- $\beta$  carbon atoms of the metallopeptide are pointed in directions other than those in natural beta-turn structure, thereby providing access to additional chemical space. The C-terminal end of the metallopeptide further provides access to a new chemical space, which space is different then that addressable by metallopeptide in **Fig. 1 A**;

**Fig. 3 A** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion superimposed on an extended chain peptide structure. In this depiction, C- $\alpha$

atoms of two consecutive amino acid residues of extended chain structure are overlapped onto the  $C_1-\alpha$  and  $C_2-\alpha$  atoms of the metallopeptide sequence. The superimposition further illustrates positioning of these two amino acid residues, including their  $C-\beta$  carbon atoms, in approximately similar chemical juxtaposition;

5        **Fig. 3 B** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion superimposed on an extended chain peptide structure. In this depiction,  $C-\alpha$  atoms of two consecutive amino acid residues of the extended chain structure are overlapped onto  $C_2-\alpha$  and  $C_3-\alpha$  atoms of the metallopeptide sequence. The superimposition illustrates exact positioning of  $C-\alpha$  carbon atoms of these two amino acid residues as well as the  $C_3-\beta$  carbon atom, while allowing access to a different chemical space at the  $C_2-\beta$  carbon atom;

10        **Fig. 4 A** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion superimposed on a  $\beta$ -sheet peptide structure. In this depiction  $C-\alpha$  atoms of two consecutive amino acid residues of the  $\beta$ -sheet structure are overlapped onto the  $C_1-\alpha$  and  $C_2-\alpha$  atoms of the metallopeptide sequence. The superimposition illustrates these two amino acid residues along with their  $C-\beta$  carbon atoms in similar chemical juxtaposition;

15        **Fig. 4 B** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion superimposed on a  $\beta$ -sheet peptide structure. In this depiction  $C-\alpha$  atoms of two consecutive amino acid residues of the  $\beta$ -sheet structure are overlapped onto the  $C_2-\alpha$  and  $C_3-\alpha$  atoms of the metallopeptide sequence. The superimposition illustrates exact positioning of  $C-\alpha$  carbon atoms of these two amino acid residues while allowing access to a different chemical space at the  $C_2-\beta$  and  $C_3-\beta$  carbon atoms;

20        **Fig. 4 C** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion superimposed on a  $\beta$ -sheet peptide structure. In this depiction  $C-\alpha$  atoms of two consecutive amino acid residues of the  $\beta$ -sheet structure are overlapped onto  $C_2-\alpha$  and  $C_3-\alpha$  atoms of the metallopeptide sequence in a manner different than that in **Fig. 4 B**. This superimposition orientation suggests positioning of these two amino acid residues along with  $C_3-\beta$  carbon atoms in similar chemical juxtaposition and allowing for accessing alternate chemical space at  $C_2-\beta$  atom;

25        **Fig. 5** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion when viewed along the plane passing through the  $C-\alpha$  carbons of amino acid residues 1, 2, 3, and D-Cys. The diagram depicts helicity in the metallopeptide with respect to amino

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acid positions 1 and 5. This  $i$  to  $i + 5$  residue pitch of the metallopeptide can be matched topographically to the  $i$  and  $i + 4$  residues chemical space in an  $\alpha$ -helix;

**Fig. 6** is a backbone diagram of the peptide sequence Ala-Ala-Ala-Cys-Ala (SEQ ID NO:1) complexed to a rhenium metal ion when viewed along the plane passing through the C-  $\alpha$  carbons of amino acid residues 1, 2, 3, and Cys. The diagram similarly depicts helicity in the metallopeptide with respect to amino acid positions 1 and 5.

**Fig. 7 A** illustrates the structure of a conceptualized L-Cys metallopeptide complexed to Re, wherein M-1 and M-2 are two amino acid residues involved in metal complexation along with the L-Cys (M-3-L) residue;

**Fig. 7 B** illustrates the structure of a conceptualized D-Cys metallopeptide complexed to Re, wherein M-1 and M-2 are two amino acid residues involved in metal complexation along with the D-Cys (M-3-D) residue;

**Fig. 8** is a phi-psi (Ramachandran) plot of the metallopeptides of **Fig. 7 A** and **7 B** showing coordinates (structural propensity) for M-1, M-2, M-3-L, and M-3-D residues. Also included in the plot are the regions of natural protein structures such as an  $\alpha$ -helix (H),  $\beta$ -sheet (B), collagen helix (C), gamma turn (G), inverse gamma turn (G-i), type-1-beta turn (I), type-1'-beta-turn (I'), type-II-beta-turn (II), type-II'-beta-turn (II'), type-III-beta-turn (III) and type-III'-beta-turn (III'). A dashed line defining an amino acid pair ( $i+1$  and  $i+2$  residues) of a turn structure is shown. The H with negative phi and psi values is for a natural right handed helix, while the other H with positive phi, psi values represents a left handed helix. The M-1 and M-2 residues reside near the  $0^\circ$ ,  $180^\circ$  or  $0^\circ$ ,  $-180^\circ$  coordinates. Both positions indicate that these amino acid residues in these metallopeptides represent a structure different than any of the natural protein structures. The phi angle in L-Cys (M-3-L) or D-Cys (M-3-D) is fixed at approximately  $-63^\circ$  and  $+63^\circ$  respectively (two solid vertical lines). Based on the psi value of Cys residues, M-3-L and M-3-D would lie somewhere on these two vertical lines. However, due to the restricted orientation of the carbonyl (CO) group of either Cys residue, the psi angle would range from  $60^\circ$  to  $90^\circ$  or  $-60^\circ$  to  $-90^\circ$  for L- and D-Cys, respectively. Under these conditions it is evident from the Ramachandran plot that the conformational characteristics at Cys fall close to a right-handed helix region for L-Cys and a left-handed helix region for D-Cys. This conclusion accords with the depictions of **Fig. 5** and **6**;



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**Fig. 9 A** illustrates the structure of an L-Cys metallopeptide complexed to Re of the primary structure des-aminoPhe-D-Asp-L-HomoSer-L-Cys-Trp-amide;

**Fig. 9 B** illustrates the structure of a D-Cys metallopeptide complexed to Re of the primary Thr-D-Lys-Gly-D-Cys-Arg;

5 **Fig. 10 A** is a circular dichroism (CD) spectra plot of the metallopeptide of **Fig. 9 A** (shown as the solid line) compared to the peptide of the structure of **Fig. 9 A** when not complexed to a metal ion (shown as the dashed line), wherein the x-plot is wavelength (nm) and the y-plot is the mean molar ellipticity  $\Theta$  of the sample per residue  $\times 10^{-3}$  (degrees  $\cdot$  cm<sup>2</sup>/decimol). The CD spectrum of the linear peptide (dashed line) shows no organized structure (zero ellipticity), whereas the CD spectrum for  
10 the Re-complexed peptide (solid line) is characteristic of ordered structure;

**Fig. 10 B** is a circular dichroism (CD) spectra plot of the metallopeptide of **Fig. 9 B** (shown as the solid line) compared to the peptide of the structure of **Fig. 9 B** when not complexed to a metal ion (shown as the dashed line), wherein the x-plot is wavelength (nm) and the y-plot is the mean molar ellipticity  $\Theta$  of the sample per residue  $\times 10^{-3}$  (degrees  $\cdot$  cm<sup>2</sup>/decimol). The CD spectrum of the linear  
15 peptide (dashed line) shows no organized structure (zero ellipticity), whereas the CD spectrum for the Re-complexed peptide (solid line) is characteristic of ordered structure; and

**Fig. 11** is a generic structure of both the urokinase-type tissue plasminogen activator metallopeptide template of Example 1 and the melanocortin metallopeptide template of Example 2.

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DESCRIPTION OF THE PREFERRED EMBODIMENTS  
(BEST MODES FOR CARRYING OUT THE INVENTION)

Certain terms as used throughout the specification and claims are defined as follows:

The terms "bind," "binding," "label," "labeling," "complex," and "complexing," as used throughout the specification and claims are generally intended to cover all types of physical and  
25 chemical binding, reactions, complexing, attraction, chelating and the like.

The "polypeptides" and "peptides" of this invention can be a) naturally-occurring, b) produced by chemical synthesis, c) produced by recombinant DNA technology, d) produced by biochemical or enzymatic fragmentation of larger molecules, e) produced by methods resulting from a combination of methods a through d listed above, or f) produced by any other means for producing polypeptides  
30 or peptides.

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The term "polypeptide" as used throughout the specification and claims is intended to include any structure comprised of two or more amino acid residues, including chemical modifications and derivatives of amino acid residues. The term "polypeptides" thus includes a conventional "peptide" containing from two to about 20 amino acid residues, a conventional polypeptide with from about 20  
5 to about 50 amino acid residues, and a conventional "protein" with a minimum of about fifty 50 amino acid residues. For the most part, the polypeptides made according to this invention and utilized as metalloptides comprise fewer than 100 amino acid residues, and preferably fewer than 60 amino acid residues, and most preferably ranging from about 3 to 20 amino acid residues. The amino acid residues forming all or a part of a polypeptide may be naturally occurring amino acid residues,  
10 stereoisomers and modifications of such amino acid residues, non-protein amino acid residues, post-translationally modified amino acid residues, enzymatically modified amino acid residues, constructs or structures designed to mimic amino acid residues, and the like, so that the term "polypeptide" includes pseudopeptides and peptidomimetics, including structures which have a non-peptidic backbone. A "manufactured" peptide or polypeptide includes a peptide or polypeptide produced by  
15 chemical synthesis, recombinant DNA technology, biochemical or enzymatic fragmentation of larger molecules, combinations of the foregoing or, in general, made by any other method.

The "amino acid residues" used in this invention, and the term as used in the specification and claims, include the known naturally occurring coded protein amino acid residues, which are referred to by both their common three letter abbreviation and single letter abbreviation. See  
20 generally Synthetic Peptides: A User's Guide, GA Grant, editor, W.H. Freeman & Co., New York, 1992, the teachings of which are incorporated herein by reference, including the text and table set forth at pages 11 through 24. As set forth above, the term "amino acid residue" also includes stereoisomers and modifications of naturally occurring protein amino acid residues, non-protein amino acid residues, post-translationally modified amino acid residues, enzymatically synthesized  
25 amino acid residues, derivatized amino acid residues, constructs or structures designed to mimic amino acid residues, and the like. Modified and unusual amino acid residues are described generally in Synthetic Peptides: A User's Guide, cited above; Hruby VJ, Al-obeidi F and Kazmierski W: *Biochem J* 268:249-262, 1990; and Toniolo C: *Int J Peptide Protein Res* 35:287-300, 1990; the teachings of all of which are incorporated herein by reference. A single amino acid residue, or a  
30 derivative thereof, is sometimes referred to herein as a "residue" or as an "amino acid."

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The constructs of this invention also include a metal ion, which may be an ionic form of any element in metallic form, including but not limited to metals and metalloids. The metal ion may, but need not, be radioactive, paramagnetic or superparamagnetic. The metal ion can be of any oxidation state of any metal, including oxidation states of vanadium (V), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), yttrium (Y), molybdenum (Mo), technetium (Tc), ruthenium (Ru), rhodium (Rh), palladium (Pd), silver (Ag), cadmium (Cd), indium (In), tin (Sn), tungsten (W), rhenium (Re), osmium (Os), iridium (Ir), platinum (Pt), gold (Au), mercury (Hg), thallium (Tl), lead (Pb), bismuth (Bi), polonium (Po), astatine (At), samarium (Sm), europium (Eu), and gadolinium (Gd). The metal ion can also be a radionuclide of any of the foregoing, including In, Au, Ag, Hg, Tc, Re, Sn, At, Y and Cu. A preferred metal ion with a tetradentate coordination sphere is Re. For applications wherein a radioisotope is desirable for screening or in assay systems, an alpha-, gamma- or beta-emitting radionuclide may be employed.

In one embodiment, the method of the invention provides for the systematic analysis of a parent polypeptide to determine at least one active sequence or domain in the parent polypeptide that is involved in the interaction, such as binding, of the parent polypeptide with a target substance. As used herein, "parent polypeptide" refers to any sequence of amino acid residues that exhibits interaction, such as binding, to a target substance, and which may thus constitute a peptide, a polypeptide or a protein. The parent polypeptide is generally a polypeptide as defined herein, with from about 3 to about 100 amino acid residues, but the term parent polypeptide can also include larger constructs, generally considered in the art to be large polypeptides or proteins. To employ the method of the invention, the primary structure, which is to say the sequence, of at least part, and preferably of all, of the parent polypeptide must be known. However, it is not necessary to have any information concerning the secondary or tertiary structure of the parent polypeptide in order to practice the method of the invention.

The parent polypeptide may be any sequence that exhibits binding to a receptor found on, for example, cells, tissues, organs or other biological materials. Examples of parent polypeptides include, without limitation, biologically active peptides, hormones, neurotransmitters, enzymes, antibodies and the like. Such parent polypeptides may transmit signals, directly or indirectly, as a result of binding to a receptor, and thus a parent polypeptide may be an agonist, an antagonist, or a mixed agonist-antagonist. Examples of suitable parent polypeptides of the invention include

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melanocortin-receptor specific peptides, urokinase-type tissue plasminogen activator protein, amyloid beta-protein related peptides, prion disease related peptides, vasopressin peptides, oxytocin peptides, angiotensin peptides, calcitonin, calcitonin gene related peptide, opioid peptides, human growth hormone, human prolactin receptor ligands, various interferons, such as alpha-interferon, 5 epidermal growth factor, tumor necrosis factor, and various hypotensive peptides, fibrinolytic peptides, chemotactic peptides, growth promoter peptides, mitogens, immunomodulators and the like.

In general, in order to employ the invention at least one assay or test to determine binding of the constructs of the invention to a receptor of interest, and preferably to also determine binding of 10 the parent polypeptide to a receptor of interest, must be known. In a preferred embodiment of the invention, a competitive inhibition or similar assay is employed, whereby the binding or functional activity of a construct of the invention can be directly compared to the parent polypeptide, and relative binding or functional activity thus directly determined. In other embodiment other assays or tests may be employed. These assays may, but need not, be functional assays. Examples of 15 assays include any of a variety of competitive inhibition assays, direct binding assays, functional assays, and the like. It is also possible and contemplated to employ assays that determine, for example, whether a construct of the invention is an agonist, antagonist or mixed agonist-antagonist, and further where binding and function can separately be determined, to independently determine both receptor affinity and specificity as well as functional activity. Examples of such assays and 20 tests are well known and well documented in the art, and in general one or more such assays or tests are known for any parent polypeptide.

In a method of the invention, the parent polypeptide is employed as the template for generation of one or more, and preferably of a series, of peptides that are then complexed to a metal ion. In general, but not necessarily, the generated peptides are of shorter length than the parent 25 polypeptide. However, it is possible and contemplated for the generated peptide to have a primary structure either as long as or longer than that of the parent polypeptide. The generated peptide, of whatever length, is complexed to a metal ion, thereby forming a metallopeptide. The metallopeptide is then employed in any of a variety of known or new assays or tests, and the binding or function, or both, of the metallopeptide compared to that of the parent polypeptide.

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The coordination sphere of various common metal ions, in general, is tetradentate to hexadentate. In one embodiment according to this invention, residues are included within each generated peptide such that the peptide contains the desired number of groups (4 to 6 in most cases) for complexing with the metal. As a result, upon complexing with a metal, the resulting metallopeptide forms a secondary structural motif about the site of metal complexation. A metal with coordination number 4, 5 or 6, and complexing respectively with an amino acid sequence forming a tetra, penta, or hexadentate ligand, will fold and constrain the ligand. The amino acid or amino acid mimetic sequence forming a ligand is defined as the metal ion-complexing domain ("MCD") of the peptide or peptidomimetic. A highly flexible molecule like a peptide, in other words, is folded to form a secondary structural motif upon its complexation with a metal ion. This resulting motif is a highly constrained structure in the conformational sense.

A binding domain ("BD") of the metallopeptide is defined in the specification and claims as a sequence of two or more amino acid residues which constitute a biologically active sequence, exhibiting binding to a receptor found on cells, tissues, organs and other biological materials, thereby constituting the metallopeptide as a member of a specific binding pair. In preferred embodiments of this invention, the BD of a metallopeptide of this invention includes at least a portion of the MCD, and may, but need not, be co-extensive with the MCD. In preferred embodiments of this invention the sequence of amino acid residues constituting the BD are thus also all or a part of the sequence of amino acid residues constituting, together with the metal ion, a secondary structural motif. The BD also includes any sequence, which may be consecutive amino acid residues or mimetics (sychnological) or non-consecutive amino acid residues or mimetics (rhegnylogical) which forms a ligand, which ligand is capable of forming a specific interaction with its acceptor or receptor. The term "receptor" is intended to include both acceptors and receptors. The receptor may be a biological receptor. The sequence or BD may transmit a signal to the cells, tissues or other materials associated with the biological receptor after binding, but such is not required. Examples include, but are not limited to, BDs specific for hormone receptors, neurotransmitter receptors, cell surface receptors, enzyme receptors and antibody-antigen systems. The BD may be either an agonist or antagonist, or a mixed agonist-antagonist. The BD may also include any ligand for site-specific RNA or DNA binding, such as sequences that may be employed as mimics of transcription and other gene regulatory proteins. The BD may also include any sequence of one or more amino acid residues or

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mimetics, or other constrained molecular regions, which exhibit binding to a biological receptor found on other peptides, on enzymes, antibodies, or other compositions, including proteinaceous compositions, which may themselves exhibit binding to another biological receptor. A peptide or peptidomimetic complexed to a metal ion with such a BD constitutes a member of a "specific binding pair," which specific binding pair is made up of at least two different molecules, where one molecule has an area on the surface or in a cavity which specifically binds to a particular spatial and polar organization of the other molecule. Frequently, the members of a specific binding pair are referred to as ligand and receptor or anti-ligand. Examples of specific binding pairs include antibody-antigen pairs, hormone-receptor pairs, peptide-receptor pairs, enzyme-receptor pairs, carbohydrate-protein pairs (glycoproteins), carbohydrate-fat pairs (glycolipids), lectin-carbohydrate pairs and the like.

Conformational constraint refers to the stability and preferred conformation of the three-dimensional shape assumed by a peptide or other construct. Conformational constraints include local constraints, involving restricting the conformational mobility of a single residue in a peptide; regional constraints, involving restricting the conformational mobility of a group of residues, which residues may form some secondary structural unit; and global constraints, involving the entire peptide structure. See generally Synthetic Peptides: A User's Guide, cited above.

The primary structure of a peptide is its amino acid sequence. The secondary structure deals with the conformation of the peptide backbone and the folding up of the segments of the peptide into regular structures such as  $\alpha$ -helices,  $\beta$ -bends, turns, extended chains and the like. For example, the local three-dimensional shape assumed by an amino acid sequence complexed to a metal ion forms a secondary structure, here called a secondary structural motif. See generally Synthetic Peptides: A User's Guide, cited above, including the text, figures and tables set forth at pages 24-33, 39-41 and 58-67. A global or tertiary structure refers to a peptide structure that exhibits a preference for adopting a conformationally constrained three-dimensional shape.

The products resulting from the methods set forth herein can be used for both medical applications and veterinary applications. Typically, the product is used in humans, but may also be used in other mammals. The term "patient" is intended to denote a mammalian individual, and is so used throughout the specification and in the claims. The primary applications of this invention involve human patients, but this invention may be applied to laboratory, farm, zoo, wildlife, pet, sport

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or other animals. The products of this invention may optionally employ radionuclide ions, which may be used for diagnostic imaging purposes or for radiotherapeutic purposes.

In one preferred embodiment of the invention, the regional secondary structure of that portion of a peptide, polypeptide, or protein, and in general any molecule or molecular structure incorporating amino acid residues or mimetics thereof, binding to any receptor or target of interest is defined by means of the methods and constructs hereafter provided. In a related preferred embodiment, the pharmacophore of a receptor or target of interest, for which there is a known peptide, polypeptide, or protein, or in general any molecule or molecular structure incorporating amino acid residues or mimetics thereof that binds thereto, is defined by means of the methods and constructs hereafter provided.

The present invention employs to advantage the unique structures and characteristics of metalloptides and similar metalloconstructs formed by complexing a metal ion to two or more amino acid residues, and in a preferred embodiment, to three amino acid residues. For most metal ions, including for example ions of Re, Tc, Cu, Ni, Au, Ag, Sn and Hg, a complex to an MCD including an available sulfur atom (S) is preferred. That is, metal ions, provided that such ions are in the appropriate and desired oxidation state for complexing, will preferably complex to a tri-peptide MCD sequence including a residue with an S available for complexing, and most preferably a residue including both an S and a nitrogen atom (N) available for complexing, in preference to tri-peptide sequences wherein no S is available for complexing.

It may thus be seen that in any amino acid sequence of length  $n$ , where  $n$  is at least 3, metal ions in the appropriate and desired oxidation state will preferentially complex to a tri-peptide sequence X-X-Cys, forming the MCD, where each X is independently any natural amino acid residue other than Pro or Cys, and further provided that the only Cys present in the amino acid sequence of length  $n$  is the Cys in X-Y-Cys. That is, the dynamics of the metal complexation reaction is such that the preferred resulting metalloptide includes, for a tetradenate metal ion, an  $N_3S_1$  ligand, formed of the tri-peptide sequence X-Y-Cys. With more than one Cys residue, or mimetic or variation of a Cys residue providing both an N and S, the structure of the resulting metalloptide is difficult to predict, and a variety of species of metalloptides may result from complexing with a metal ion. For example, an amino acid sequence of length  $n$  containing two Cys residues may cross-link, dimerize, polymerize, form internal disulfide bridges and the like. In terms of metal complexation, there may,

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depending on the primary structure of the sequence, be two different MCDs, such that some molecules will have a metal ion bound to the first MCD, others to the second MCD and still others to both MCDs. Thus the structure of a resulting metallopeptide cannot be predicted, and must be empirically determined. Similarly, it is also possible, again depending on the primary structure of the sequence, that one or more MCDs in the sequence will provide  $N_3S_1$  ligands, while at least one other MCD in the sequence will provide an  $N_2S_2$  ligand. Here too the structure of the resulting metallopeptide cannot be predicted, and must be empirically determined.

The present invention encompasses a method for defining the secondary structure of a region of a peptide, polypeptide, or protein, or in general any molecule or molecular structure incorporating amino acid residues or mimetics thereof, that binds to any receptor or target of interest. This is accomplished by substitution or insertion of a Cys residue, or other residue, mimetic, or homologue providing both an N and S for complexation to the coordination sphere of a metal ion (an " $N_1S_1$  residue"), at various positions along the molecule, complexing a metal ion thereto to form a metallopeptide, and testing the resulting metallopeptide for binding to the receptor or target of interest. In one embodiment of the invention, the primary structure of a parent polypeptide, such as a peptide, polypeptide or protein binding to a receptor or target of interest, is known. Such parent polypeptide is composed of some specific number of residues, referred to as "n" residues. A series of peptides of the formula  $R_1-C-R_2$  is made, wherein  $R_1$  is from 2 to n residues that are the same as or homologues of residues in the parent polypeptide and in the same order as in the parent polypeptide. C is any  $N_1S_1$  residue, including but not limited to L-Cys, D-Cys, L-Pen, D-Pen or 3-mercapto phenylalanine.  $R_2$  is from 0 to n-2 different residues that are the same as, or homologues of, residues in the known primary structure in the same order as in the parent polypeptide. Further,  $R_1$  and  $R_2$  together constitute at least two residues, and together form a sequence in the same order as in the parent sequence where C is either inserted between two adjacent residues or substitutes for a single residue. Any Cys in  $R_1$  or  $R_2$  may be conservatively substituted with Gly, Ala or Ser (among naturally occurring coded protein amino acid residues), and preferably Gly or Ala. Alternatively, a Cys with an S-protecting group (as hereafter described) may be employed. In a further embodiment, any synthetic or unnatural relatively small, neutral amino acid may be employed, for example amino isobutyric acid (Aib) or dehydroalanine ( $\Delta$ Ala). Any Pro in the two residues on the immediately adjacent amino-terminus side of C is located in a position that forms a part of the



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putative MCD, and is similarly conservatively substituted. Such substitution is required because there is no available N in Pro to complex to the coordination sphere of a metal ion, and therefore Pro cannot form a part of the MCD. Accordingly, any such Pro may be substituted with Gly, Ala or Ser (among naturally occurring coded protein amino acid residues), and preferably Gly or Ala. In a further embodiment, any synthetic or unnatural relatively small, neutral amino acid may be employed, for example Aib or  $\Delta$ Ala.

In the specification and the claims, the term "homologue" includes, in the case of a Cys to be substituted as set forth above, a conservative substitution with Gly, Ala or Ser, and preferably Gly or Ala. The term "homologue" further includes a Cys with an S-protecting group, wherein because of the S-protecting group the sulfur in the Cys residue is no longer available for binding to a metal ion. The terms "homologue" further includes, in the case of a Cys to be substituted, any synthetic or unnatural relatively small, neutral amino acid, for example Aib or  $\Delta$ Ala. In the case of a Pro to be substituted as set forth above, the term "homologue" includes a conservative substitution with Gly, Ala or Ser, and preferably Gly or Ala. The terms "homologue" further includes, in the case of a Pro to be substituted, any synthetic or unnatural relatively small, neutral amino acid, for example Aib or  $\Delta$ Ala. In the case of residues in either R<sub>1</sub> or R<sub>2</sub>, other than Pro in the two residues on the immediately adjacent amino-terminus side of C or Cys, a "homologue" of such residue includes (a) a D-amino acid residue substituted for an L-amino acid residue, (b) a post-translationally modified residue, (c) a non-protein amino acid or other modified amino acid residue based on such residue, such as phenylglycine, homophenylalanine, ring-substituted halogenated, and alkylated or arylated phenylalanines for a phenylalanine residue, diamino proionic acid, diamino butyric acid, ornithine, lysine and homoarginine for an arginine residue, and the like, and (d) any amino acid residue, coded or otherwise, or a construct or structure that mimics an amino acid residue, which has a similarly charged side chain (neutral, positive or negative), preferably a similar hydrophobicity or hydrophilicity, and preferably a similar side chain in terms of being a saturated aliphatic side chain, a functionalized aliphatic side chain, an aromatic side chain or a heteroaromatic side chain.

Assume, for example, a parent polypeptide of six amino acid residues or residues that binds to a specified and known receptor. The parent polypeptide may be described as:



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Employing the formula  $R_1-C-R_2$  as defined above, and assuming, for example, that in the first instance a Cys is used for C and is inserted between the  $X^4$  and  $X^5$  positions, it can be seen that the following peptides are contemplated by the invention with respect to insertion of Cys between the  $X^4$  and the  $X^5$  positions:

- 5             $X^1-X^2-X^3-X^4-Cys-X^5-X^6$   
               $X^2-X^3-X^4-Cys-X^5-X^6$   
               $X^3-X^4-Cys-X^5-X^6$   
               $X^1-X^2-X^3-X^4-Cys-X^5$   
               $X^1-X^2-X^3-X^4-Cys$   
 10            $X^2-X^3-X^4-Cys-X^5$   
               $X^2-X^3-X^4-Cys$   
               $X^3-X^4-Cys-X^5$   
               $X^3-X^4-Cys$

- Similar series of peptides can be generated assuming that the Cys is inserted between the  $X^2$  and  $X^3$  positions, between the  $X^3$  and  $X^4$  positions, between the  $X^5$  and  $X^6$  positions, or following the  $X^6$  position. For example, assuming that Cys is inserted between the  $X^2$  and  $X^3$  positions, the following peptides result:

- $X^1-X^2-Cys-X^3-X^4-X^5-X^6$   
               $X^1-X^2-Cys-X^3-X^4-X^5$   
 20            $X^1-X^2-Cys-X^3-X^4$   
               $X^1-X^2-Cys-X^3$   
               $X^1-X^2-Cys$

Assuming that Cys is inserted following the  $X^6$  position the following peptides result:

- $X^1-X^2-X^3-X^4-X^5-X^6-Cys$   
 25            $X^2-X^3-X^4-X^5-X^6-Cys$   
               $X^3-X^4-X^5-X^6-Cys$   
               $X^4-X^5-X^6-Cys$   
               $X^5-X^6-Cys$

- In the practice of the invention, it is also possible and contemplated that the Cys may be employed to replace a residue in the parent polypeptide rather than be inserted between two

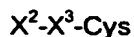
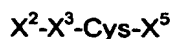
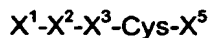
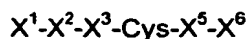
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adjacent residues. Assume again a parent polypeptide of six amino acid residues or residues that binds to a specified and known receptor described as:



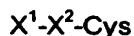
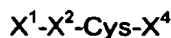
Employing the formula  $R_1-C-R_2$ , and assuming, for example, that the C is Cys and replaces, in the

- 5 first instance, the  $X^4$  residue, it can be seen that the following peptides are contemplated by the invention with respect to replacement of  $X^4$  by Cys:

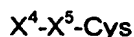
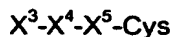
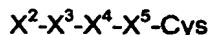
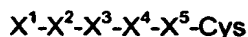


Similar series of peptides can be generated assuming that the Cys replaces the  $X^3$  residue,  $X^5$  residue or the  $X^6$  residue. For example, assuming that Cys replaces the  $X^3$  residue, the following

- 15 peptides result:



- 20 Assuming that Cys replaces the  $X^6$  residue the following peptides result:



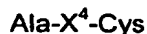
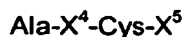
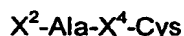
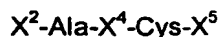
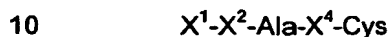
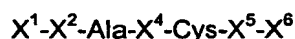
- 25 Of course, in each of the preceding examples if any of the residues in the parent polypeptide  $X^1-X^2-X^3-X^4-X^5-X^6$  are a Cys, then a conservative substitution with Gly, Ala or Ser, and preferably Gly or Ala, may be employed. Alternatively, a Cys with an S-protecting group, as hereafter described, may be employed. In a further embodiment, any synthetic or unnatural relatively small, neutral amino acid may be employed in lieu of the Cys. Assume, for example, the parent polypeptide may
- 30 be described as:

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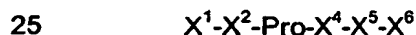
Employing the formula  $R_1-C-R_2$ , and assuming for example that in the first instance C is a Cys inserted between the  $X^4$  and  $X^5$  positions, and that Ala is employed to substitute for the endogenous Cys in the parent polypeptide in the  $X^3$  position, it can be seen that the following peptides are

5 contemplated by the invention:

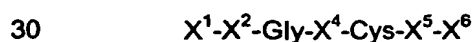


15 Similar substitutions are employed in the event that the C of the formula  $R_1-C-R_2$  is employed by replacement rather than insertion.

Similarly, if in any of the preceding examples there is a Pro in the parent polypeptide, then when that Pro is located in the sequence wherein it would be within the sequence forming a part of the MCD (i.e., the C of the formula  $R_1-C-R_2$  and the two residues immediately adjacent the amino-terminus side of C and comprising at least a part of  $R_1$ ), then a conservative substitution with Gly, Ala or Ser, and preferably Gly or Ala may be employed in lieu of Pro. Alternatively, any synthetic or unnatural relatively small, neutral amino acid or mimetic (preferably but not necessarily also hydrophobic) may be employed in lieu of Pro, on the proviso that an N is available for complexing the metal ion. Assume, for example, the parent polypeptide may be described as:



Employing the formula  $R_1-C-R_2$ , and assuming for example that in the first instance C is Cys and the Cys is inserted between the  $X^4$  and  $X^5$  positions, and that Gly is employed to substitute for the endogenous Pro in the parent polypeptide in the  $X^3$  position, it can be seen that the following peptides are contemplated by the invention:



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X<sup>2</sup>-Gly-X<sup>4</sup>-Cys-X<sup>5</sup>-X<sup>6</sup>Gly-X<sup>4</sup>-Cys-X<sup>5</sup>-X<sup>6</sup>X<sup>1</sup>-X<sup>2</sup>-Gly-X<sup>4</sup>-Cys-X<sup>5</sup>X<sup>1</sup>-X<sup>2</sup>-Gly-X<sup>4</sup>-Cys5 X<sup>2</sup>-Gly-X<sup>4</sup>-Cys-X<sup>5</sup>X<sup>2</sup>-Gly-X<sup>4</sup>-CysGly-X<sup>4</sup>-Cys-X<sup>5</sup>Gly-X<sup>4</sup>-Cys

However, in the parent polypeptide X<sup>1</sup>-X<sup>2</sup>-Pro-X<sup>4</sup>-X<sup>5</sup>-X<sup>6</sup> and assuming that Cys is inserted following  
 10 the X<sup>6</sup> position, no substitution of Pro is required, such that the following peptides result:

X<sup>1</sup>-X<sup>2</sup>-Pro-X<sup>4</sup>-X<sup>5</sup>-X<sup>6</sup>-CysX<sup>2</sup>-Pro-X<sup>4</sup>-X<sup>5</sup>-X<sup>6</sup>-CysPro-X<sup>4</sup>-X<sup>5</sup>-X<sup>6</sup>-CysX<sup>4</sup>-X<sup>5</sup>-X<sup>6</sup>-Cys15 X<sup>5</sup>-X<sup>6</sup>-Cys

Similar substitutions are employed in the event that the C of the formula R<sub>1</sub>-C-R<sub>2</sub> is employed by  
 replacement rather than insertion.

In yet another embodiment the parent polypeptide may be treated as a single unit. Assume a  
 peptide of fifteen amino acid residues or residues binds to a specified known receptor. The peptide  
 20 may be described as:

NH<sub>2</sub>-X<sup>1</sup>-X<sup>2</sup>-X<sup>3</sup>-X<sup>4</sup>-X<sup>5</sup>-X<sup>6</sup>-X<sup>7</sup>-X<sup>8</sup>-X<sup>9</sup>-X<sup>10</sup>-X<sup>11</sup>-X<sup>12</sup>-X<sup>13</sup>-X<sup>14</sup>-X<sup>15</sup>-COOH

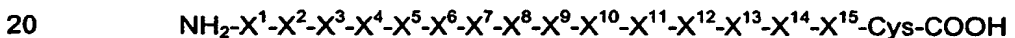
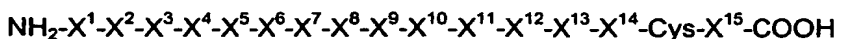
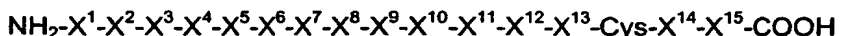
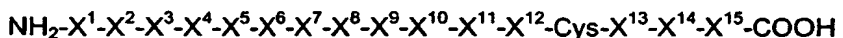
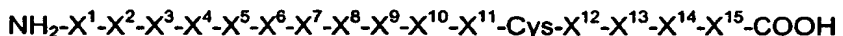
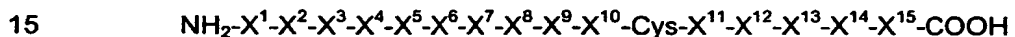
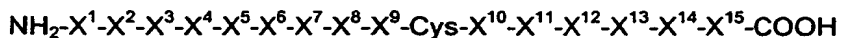
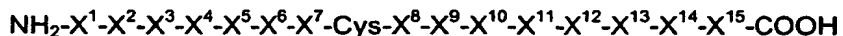
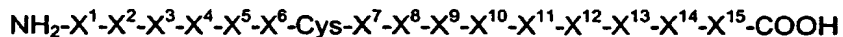
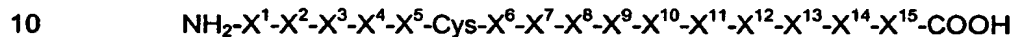
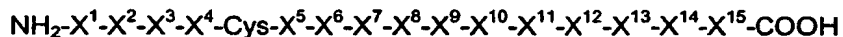
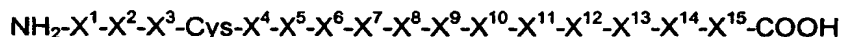
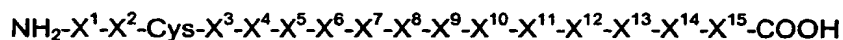
In this parent polypeptide, X may be any residue, which residue may repeat multiple times in  
 any order or sequence. Thus the residue in position X<sup>1</sup> may be different from or the same as the  
 residue in position X<sup>2</sup>, which may be different from or the same as the residues in position X<sup>1</sup> or X<sup>3</sup>,  
 25 and so on. Here too when a Cys is present in the parent polypeptide, substitution may be made.  
 Similarly, where a Pro is present that comprises a part of the putative MCD, such as when a Pro falls  
 within the two residues in R<sub>1</sub> immediately adjacent the amino-terminus side of the N<sub>1</sub>S<sub>1</sub> residue  
 hereafter provided, substitution may be made.

In the practice of this invention, an N<sub>1</sub>S<sub>1</sub> residue, providing both an N and an S for complexing  
 30 to a metal ion, is employed, such as L- or D-cysteine, or any other natural, unnatural or synthetic

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amino acid or mimetic providing both an N and S for complexing to a metal ion. For the following examples, "Cys" is employed, it being understood that any N<sub>1</sub>S<sub>1</sub> residue could be similarly employed, and that this example and those that follow are not limited to Cys as the N<sub>1</sub>S<sub>1</sub> residue. Peptides are constructed using standard peptide synthesis techniques, in which the cysteine is inserted after the

5 2nd position (X<sup>2</sup>) through the 16th (n+1) position (following X<sup>15</sup>), such that the following peptides result:



In this way each potential insertion point along the parent polypeptide is "scanned" to determine if creation of a metal ion-stabilized secondary structural motif at each insertion point results in a metallopeptide with biological activity, however defined, and preferably biological activity at least equal or approximately equal to that of the parent polypeptide.

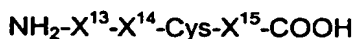
25 During synthesis the -SH group of C of the formula R<sub>1</sub>-C-R<sub>2</sub> may be protected using an orthogonal protecting agent as set forth below. The resulting orthogonally-protected Cys-containing peptide is then deprotected, and subsequently complexed with a metal ion, such as a rhenium ion, thereby forming a metallopeptide, using in the case of a rhenium ion a suitable pre-formed metal-oxo transfer agent, such as Re(O)Cl<sub>3</sub>(PPh<sub>3</sub>)<sub>2</sub>. Through use of suitable assays or tests, such as

30 competitive inhibition assays, the binding of each of the resulting metallopeptides is compared

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against the parent polypeptide, and those metallopeptides with enhanced or increased binding are identified as involving a reverse turn structure about the metal ion complex forming all or a part of the BD of the metallopeptide.

In a related approach, sequences of defined length but less than that of the parent polypeptide are synthesized. These sequences are based on, for example, the hypothetical known parent polypeptide of 15 residues as set forth above. In one embodiment, a Cys is inserted into the sequence of defined length. Thus it is possible and contemplated that a series of sequences of four amino acid residues is synthesized and screened as set forth above. The four amino acid residues consist of one residue on the carboxyl terminus side of the Cys and two residues on the amino terminus side of the Cys, as follows:



In yet another related approach, sequences of defined length but less than that of the parent polypeptide are synthesized, with the Cys employed as substitute for an amino acid residue in the parent polypeptide. Thus it is possible and contemplated that a sequence of four amino acid residues is synthesized and screened as set forth above, the four amino acid residues comprising one residue on the carboxyl terminus side of the Cys, and two residues on the amino terminus side of the Cys, with the Cys substituted for a residue in the parent polypeptide, as follows:



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NH<sub>2</sub>-X<sup>2</sup>-X<sup>3</sup>-Cys-X<sup>5</sup>-COOH  
 NH<sub>2</sub>-X<sup>3</sup>-X<sup>4</sup>-Cys-X<sup>6</sup>-COOH  
 NH<sub>2</sub>-X<sup>4</sup>-X<sup>5</sup>-Cys-X<sup>7</sup>-COOH  
 NH<sub>2</sub>-X<sup>5</sup>-X<sup>6</sup>-Cys-X<sup>8</sup>-COOH  
 5 NH<sub>2</sub>-X<sup>6</sup>-X<sup>7</sup>-Cys-X<sup>9</sup>-COOH  
 NH<sub>2</sub>-X<sup>7</sup>-X<sup>8</sup>-Cys-X<sup>10</sup>-COOH  
 NH<sub>2</sub>-X<sup>8</sup>-X<sup>9</sup>-Cys-X<sup>11</sup>-COOH  
 NH<sub>2</sub>-X<sup>9</sup>-X<sup>10</sup>-Cys-X<sup>12</sup>-COOH  
 NH<sub>2</sub>-X<sup>10</sup>-X<sup>11</sup>-Cys-X<sup>13</sup>-COOH  
 10 NH<sub>2</sub>-X<sup>11</sup>-X<sup>12</sup>-Cys-X<sup>14</sup>-COOH  
 NH<sub>2</sub>-X<sup>12</sup>-X<sup>13</sup>-Cys-X<sup>15</sup>-COOH

In yet another related approach, alternate sequences of defined length but less than that of the parent polypeptide are synthesized. Thus it is possible and contemplated that yet another sequence of four amino acid residues is synthesized and screened as set forth above, the four amino acid residues including Cys as the carboxyl-terminus residue of a tetrapeptide sequence including

15 three ordered residues from the parent polypeptide, as follows:

NH<sub>2</sub>-X<sup>1</sup>-X<sup>2</sup>-X<sup>3</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>2</sup>-X<sup>3</sup>-X<sup>4</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>3</sup>-X<sup>4</sup>-X<sup>5</sup>-Cys-COOH  
 20 NH<sub>2</sub>-X<sup>4</sup>-X<sup>5</sup>-X<sup>6</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>5</sup>-X<sup>6</sup>-X<sup>7</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>6</sup>-X<sup>7</sup>-X<sup>8</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>7</sup>-X<sup>8</sup>-X<sup>9</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>8</sup>-X<sup>9</sup>-X<sup>10</sup>-Cys-COOH  
 25 NH<sub>2</sub>-X<sup>9</sup>-X<sup>10</sup>-X<sup>11</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>10</sup>-X<sup>11</sup>-X<sup>12</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>11</sup>-X<sup>12</sup>-X<sup>13</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>12</sup>-X<sup>13</sup>-X<sup>14</sup>-Cys-COOH  
 NH<sub>2</sub>-X<sup>13</sup>-X<sup>14</sup>-X<sup>15</sup>-Cys-COOH



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In each of the foregoing examples a tetrapeptide sequence is employed, wherein one of the residues is Cys. However, it is to be understood that the sequence may be of any length from a tripeptide (e.g., X-X-Cys) to a peptide of length  $n+1$ , where  $n$  is the length of the parent polypeptide. In alternative embodiments, other residues, mimics, terminal groups can be added, such that the length of the sequence is in excess of  $n+1$ . Similarly, it is to be understood that Cys may be any residue, natural or unnatural, or mimetic thereof, or different construct, provided only that it comprises an  $N_1S_1$  residue. In each such case, the resulting Cys-containing peptides are complexed with a metal ion, such as a rhenium ion, forming a metallopeptide, such as by using a suitable pre-formed metal-oxo transfer agent such as  $Re(O)Cl_3(PPh_3)_2$ .

In yet another embodiment, it is contemplated and to be understood that a parent polypeptide may be divided into overlapping sequences, and that each such sequence is then effectively considered and treated as an independent parent polypeptide, according to the methods and constructs of this invention. For example, assume a parent polypeptide of length  $n$  where  $n$  is 30. Such parent polypeptide may be suspected of containing more than one discrete BD. Accordingly, in one embodiment the primary sequence is divided into constructs, such as three constructs. For example, one construct may consist of the residues from the 1 to 15 positions, a second the residues from the 7 to 21 positions, and a third the residues from the 16 to 30 positions. In this way, all possible endogenous and contiguous BDs are included in at least one of the three constructs. Each construct is thereafter treated as an independent parent polypeptide, according to the methods of this invention. In a preferred embodiment, this method is employed with parent polypeptides of at least a length where  $n$  is 15, with three divided constructs employed, each such divided construct overlapping the adjacent divided construct by at least two residues.

Through use of suitable screen assays, such as competitive inhibition assays, the binding of each of the resulting metallopeptides is compared against the parent polypeptide, and those with enhanced or increased binding are identified as involving a secondary structural motif about the metal ion complex forming at least a part of the BD. Once one or more metallopeptides with enhanced or increased binding are identified, amino acid residues on either the amino or carbonyl ends may be added, subtracted, and the like, side chains modified, and similar changes made to obtain a metallopeptide with optimal binding or other desired characteristics, including agonist, antagonist or mixed agonist/antagonist activity.

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In the event that the parent polypeptide contains one or more endogenous Cys residues, it is possible to protect the intrinsic Cys residues with a non-orthogonal -SH protecting agent, to protect the introduced N<sub>1</sub>S<sub>1</sub> residue with an orthogonal -SH protecting agent, to thereafter selectively deprotect the orthogonal -SH protecting agent, to then complex the deprotected N<sub>1</sub>S<sub>1</sub> residue with a metal ion, and thereafter to deprotect the Cys residue with the non-orthogonal -SH protecting agent. Examples of common non-orthogonal -SH protecting groups include, but are not limited to, trityl, benzyl, p-methoxy benzyl, and <sup>t</sup>Bu.

It may further be seen from the foregoing that in another embodiment of the invention the pharmacophore of a receptor or other target of interest may be defined. Assume that a known parent polypeptide (which may be a peptide, polypeptide or protein), binds to a receptor for which definition of the pharmacophore is desired. While the primary structure of the parent polypeptide is known, the specific residues involved in binding to the receptor, and the secondary structure involved in such binding to the receptor, is not known. Thus definition of the pharmacophore cannot be derived solely from knowledge of the primary structure of the parent polypeptide. Knowledge of the pharmacophore may, for example, permit design and construction of any of a wide variety of small molecules, including peptidomimetics and non-peptide small molecules, which bind to the receptor, optionally acting as either an agonist or antagonist. Based on the primary structure of the known parent polypeptide, a series of metallopeptides is constructed as set forth above. The metallopeptide with optimal binding and other desired characteristics with respect to the receptor and the parent polypeptide is selected. The selected metallopeptide may be optimized as desired, such as by determining the fewest residues yielding acceptable binding, for example such that in the formula R<sub>1</sub>-Cys-R<sub>2</sub>, R<sub>1</sub> and R<sub>2</sub> together constitute no more than three, and optionally preferably only two, residues. Similarly, modifications to optimize the selected metallopeptide may optionally be made with respect to side chains, such that the resulting metallopeptide has desired hydrogen bond donors and acceptors, charged centers, aromatic ring centers, hydrophobic centers and the like, thereby providing optimal binding to the receptor.

When a metallopeptide is selected that provides optimal binding to the desired receptor compared to the parent polypeptide, as determined by the methods of this invention, then the metallopeptide so selected may be modeled. In a typical peptide (i.e. a parent polypeptide), there are a wide variety of torsion angles that determine a diverse range of probabilistically-determined

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secondary and tertiary structures of the peptide. Thus with a typical peptide, knowledge of the primary structure does not necessary imply that the secondary or tertiary structure can be determined absent empirical evidence. However, with a metallopeptide of this invention, employing the formula  $R_1\text{-Cys-R}_2$ , the metal ion and MCD of the metallopeptide are conformationally constrained, with a fixed and determined secondary structure. Because of the metal ion complexation, the torsion angles within and between the residues of the MCD are fixed and may be determined based upon the type of metal ion employed, including its oxidation state, coordination geometries and the like.

As a result, any metallopeptide, including specifically the portion thereof the MCD and, to a significant extent, residues adjacent to the MCD, may be modeled, thereby determining the secondary structure. By this means the pharmacophore can be modeled as the complement to the metallopeptide. For example, the location in a three-dimensional construct of hydrogen bond donors and acceptors, positively and negatively charged centers, aromatic ring centers, hydrophobic centers and the like may be determined (including determination of the distance between atoms constituting a part of the pharmacophore). Any of a wide variety of software programs may be employed for such modeling, including programs such as SYBYL (Tripos, Inc.), Alchemy (Tripos, Inc.), Align/Pharmacophore (Accelrys Inc.), Catalyst (Accelrys Inc.), MacroModel (Schrödinger, Inc.), PC-Model (Serena Software), CS ChemOffice (CambridgeSoft Corporation) and other programs known in the field. Techniques for pharmacophore modeling are taught in any number of articles and texts, including Pharmacophore Perception, Development and Use in Drug Design, Osman F. Güner, Ed., Int'l University Line, La Jolla, CA, 2000; and Guidebook on Molecular Modeling in Drug Design, N. Claude Cohen, Ed., Academic Press, San Diego, 1996.

It may further be seen that using the methods and constructs of this invention libraries of metallopeptides may be designed and made wherein each constituent series member includes an MCD sequence necessary for providing a coordination site for complexation with a metal. These libraries may be made using any method, including specifically solution and solid phase synthesis techniques.

Upon complexing the MCD with a metal, a specific structure results which forms a secondary structural motif. The specific stereochemical features of this complex are due to the stereochemistry of the coordination sphere of the complexing metal ion. The preferred geometry of the coordination

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sphere of the metal dictates and defines the nature and extent of conformational restriction. In general, most of the metals that may prove useful in this invention have a coordination number of 4 to 6 (and sometimes, but rarely, as high as 8), which implies that the putative MCD must be made of residues with reactive groups located in a stereocompatible manner establishing a bond with a metal ion of given geometry and coordination sphere. Coordinating groups in the peptide chain include nitrogen atoms of amine, amide, imidazole, or guanidino functionalities; sulfur atoms of thiols or disulfides; and oxygen atoms of hydroxy, phenolic, carbonyl, or carboxyl functionalities. In addition, the peptide chain or individual amino acid residues can be chemically altered to include a coordinating group, such as oxime, hydrazino, sulfhydryl, phosphate, cyano, pyridino, piperidino, or morpholino groups. For a metal with a coordination number of 4, a preferred MCD is a three amino acid sequence in which one of the amino acid residues has a side chain with a sulfur-based coordinating group (such as Cys), such residue constituting an  $N_1S_1$  ligand. Thus, a three amino acid sequence can provide an  $N_3S$ ,  $N_2SO$  or similar ligand, yielding tetradentate coordination of a metal ion utilizing nitrogen and sulfur and, optionally, oxygen atoms.

The choice of metal ion partially determines the structure of the resulting turning structure. For example, use of an Re ion results in a square pyramidal coordination geometry. Tc (which has substantially similar coordination requirements and chemistries and generally may be substituted for Re in any example herein) similarly results in a square pyramidal coordination geometry. Use of other metal ions, such as Cu, Ni or Zn, results in square planar coordination geometries. Thus while the atomic radius of Re is on the order of 1.37 Å and that of Cu is smaller, on the order of 1.28 Å, the resulting dimensions of the metal coordination group is determined, in large part, by the coordination geometry, and not just by the atomic radius of the metal ion. With metal ions such as Cu, Ni or Zn employing square planar coordination tetradentate geometries, the metal ion and each of the four coordinating atoms (such as S, N or O) are co-planar. However, when employing metal ions such as Re or Tc (which result in square pyramidal coordination tetradentate geometries), the four coordinating atoms (such as S, N or O) are co-planar, but the metal ion is, in the case of Re, about .65 Å removed from the plane of the coordinating atoms.

In this invention any of a wide range of metal ions may be employed, but Re and Tc are particularly preferred. Both metals form similar complexes with Cys-containing peptides yielding similar square pyramidal complexes. Re-complexed peptides, however, are chemically more stable

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than the corresponding Tc-containing peptides. The square planar complexes of Zn and Cu, with the metal ion as well as the four coordinating atoms of the peptide all in one plane, results in a near identical complexation geometry as is obtained with Tc or Re, where the metal ion is projected upwards from the plane of four coordinating atoms of the peptide, notwithstanding the differences in the atomic radius of the metal ions. The net result are metallopeptides that each afford topographic similarities, whether for example Re, Tc, Zn or Cu is employed. The Re-complexed metallopeptides, however, are unique in that the metallopeptides are air and moisture stable, without any need for special or exotic excipients or protecting agents. The Re-complexes can routinely be isolated as solid compounds and are stable as solids and in solutions over a wide pH range, thereby facilitating both analytical characterization and, more importantly, use in both in vitro and in vivo biological experiments over a wide range of conditions. Other metal types, such as Zn-complexes and Cu-complexes, are utilized in experiments in a solution form. However, Zn-complexes and Cu-complexes are extremely easy to form, and essentially are formed in the presence of 1 micromolar to 1 millimolar concentration of the metal ion in an appropriately buffered solution.

The Re- and Tc-complexes are metaloxo complexes, generally and in a preferred embodiment in an oxidation state [V]. The metaloxo core M=O in the metallopeptides may give rise to an isomerism in the core structure. The metal-oxo group may be *syn* or *anti* with respect to a chiral amino acid side chain. Since the orientation of the oxo group does not alter the topographic surface created by the amino acid side chains, this isomerism has no effect on the biological activity of the metallopeptides. It can be well appreciated from Figs. 1 A, 1 C, 2 A, 2 B, 3 A, 3 B, 4 A, 4 B, 4 C, 5 and 6 that the oxo group of a metal ion does not sterically hinder the conformationally constrained amino acid side chain presentations. In fact, the metal ion is situated at a location spatially similar to that where turns are stabilized by a hydrogen bond in natural turn structures; thus the oxo group falls within a space not addressable in natural turn structures. The computer modeling of individual *syn*- and *anti*-isomers of metallopeptides have shown that these two structures are completely indistinguishable with respect to each amino acid location, with orientation of the oxo group being the only difference.

The utility of an embodiment of the invention, resulting in a structure that mimics topologies of naturally occurring peptide and protein structures, may be perceived with reference to certain of the figures of the invention. Protein structure is discussed and explain extensively in Introduction to

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Protein Structure, Carl Branden and John Tooze, 1991, Garland Publishing Inc. New York and London, and the discussion therein is incorporated here by reference. **Fig. 1 B** depicts the backbone structure of a classical beta-II' turn. **Fig. 1 A** depicts the structure, of an Re metal-coordinated pentapeptide of SEQ ID NO:1, wherein an L-Cys is employed. **Fig. 1 C** is the diagram of **Fig. 1 A** superimposed on the diagram of **Fig. 1 B** at their respective C- $\alpha$  carbon atoms of the three consecutive N-terminal amino acid residues (C<sub>1</sub>- $\alpha$ , C<sub>2</sub>- $\alpha$  and C<sub>3</sub>- $\alpha$  atoms). It can be seen by examination of **Fig. 1 C** that there is excellent overlap at these three carbon atoms, with an RMSD < 0.05 Å, demonstrating that the turn structure of **Fig. 1 A** forms a close mimic of the classical beta-II' turn of **Fig. 1 B**. Thus the topology and relative relationship of, for example, side chains of these amino acid residues of **Fig. 1 A** and **Fig. 1 B** would be very similar. It should be noted that the sequence employed for **Fig. 1 A**, Ala-Ala-Ala-Cys-Ala (SEQ ID NO:1), was employed only as a model, and that any pentapeptide wherein Cys is the 4 position and the remainder of the residues are any residue other than Cys or Pro would result in a similar backbone diagram, with the same overlap of the C<sub>1</sub>- $\alpha$ , C<sub>2</sub>- $\alpha$  and C<sub>3</sub>- $\alpha$  atoms.

**Fig. 2 A** depicts the structure, similarly by way of a backbone diagram, of a Re metal-coordinated Ala-Ala-Ala-D-Cys-Ala pentapeptide. **Fig. 2 B** is a diagram of **Fig. 2 A** superimposed at the respective C- $\alpha$  carbon atoms of the three consecutive N-terminal amino acid residues (C<sub>1</sub>- $\alpha$ , C<sub>2</sub>- $\alpha$  and C<sub>3</sub>- $\alpha$  atoms). It can be seen by examination of **Fig. 1 C** that there is here also excellent overlap at these three carbon atoms, similarly with an RMSD < 0.05 Å, demonstrating that the turn structure of **Fig. 2 A** also forms a close mimic of the classical beta-II' turn of **Fig. 1 B**. Thus the topology and relative relationship of, for example, side chains of amino acid residues of **Fig. 2 A** and **Fig. 1 B** would be very similar. Here too any pentapeptide sequence employing a D-Cys in the 4 position and any residues other than Cys or Pro in the remaining positions would result in a similar backbone diagram, with the same overlap of the C<sub>1</sub>- $\alpha$ , C<sub>2</sub>- $\alpha$  and C<sub>3</sub>- $\alpha$  atoms. It is also evident from a comparison of Re-peptide structures in **Fig. 1 A** and **2 A** that the C-terminal 5th amino acid extensions in these templates effectively allow for accessing additional and distinct chemical space for establishing a specific receptor contact, thereby adding to enhanced diversity in these structures.

**Fig. 3 A** is a backbone diagram of the peptide sequence Ala-Ala-Ala-D-Cys-Ala complexed to a rhenium metal ion superimposed on an extended chain peptide structure. In this depiction C- $\alpha$  atoms of two consecutive amino acid residues of extended chain structure are overlapped onto C<sub>1</sub>- $\alpha$

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and C<sub>2</sub>-α atoms of the metallopeptide sequence. The superimposition suggests positioning of these two amino acid residues along with their C-beta carbon atoms in approximately similar chemical juxtaposition. **Fig. 3 B** similarly depicts C-α atoms of two consecutive amino acid residues of an extended chain structure overlapped onto the C<sub>2</sub>-α and C<sub>3</sub>-α atoms of the sequence Ala-Ala-Ala-D-Cys-Ala. The superimposition suggests exact positioning of C<sub>2</sub>-α and C<sub>3</sub>-α atoms as well as the C<sub>3</sub>-β carbon atom, while allowing access to a different chemical space at the C<sub>2</sub>-β carbon atom;

**Figs. 4 A, 4 B and 4 C** illustrate the rhenium complexed peptide sequence Ala-Ala-Ala-D-Cys-Ala superimposed on a β-sheet peptide structure. Three separate superimpositions are shown: that of **Fig. 4 A** with C-α atoms of two consecutive amino acid residues of the β-sheet structure overlapped onto C<sub>1</sub>-α and C<sub>2</sub>-α atoms of the metallopeptide sequence; that of **Fig. 4 B** with C-α atoms of two consecutive amino acid residues of the β-sheet structure overlapped onto C<sub>2</sub>-α and C<sub>3</sub>-α atoms of the metallopeptide; and that of **Fig. 4 C** with C-α atoms of two consecutive amino acid residues of the β-sheet structure overlapped onto C<sub>2</sub>-α and C<sub>3</sub>-α atoms of the metallopeptide in a different orientation that as shown in **Fig. 4 B**. Each illustrates either similar or exact positioning of C-α carbon atoms, while allowing the metalloptides to access additional or different chemical space, such as at the C<sub>2</sub>-β and C<sub>3</sub>-β carbon atoms in the case of **Fig. 4 B** or at the C<sub>2</sub>-β atom in the case of **Fig. 4 C**.

**Fig. 5** illustrates that the topology of side chains in a metallopeptide can be organized and selected similar to that observed in natural turn structures, such as helices. Thus there is a functional helicity in the metallopeptide with respect to amino acid residues 1 and 5, which i to i + 5 residue pitch on the metallopeptide can be matched topographically to the i and i + 4 residues chemical space in an α-helix. Similarly, utilizing a natural Cys, a similar topology results, as shown in **Fig. 6**.

The Ramachandran plot of **Fig. 8** shows the coordinates, and thus corresponding structural propensity, of the M-1, M-2, M-3-L, and M-3-D residues of the metalloptides of **Fig. 7 A and 7 B**. It can thus be seen that a metallopeptide with an L-Cys forms a mimic of a short right hand turn of helix, while a metallopeptide with a D-Cys forms a mimic of a short left hand turn of a helix. It is well known that natural helix turns are right handed only. The metallopeptide approach, therefore, offers the advantage that both right and left handed structures can be constructed. These structure can be utilized to topographically position the side chains of i and i + 5 residues in a L-Cys containing

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metallopeptide in the same chemical space as that for the side chains of  $i$  and  $i + 4$  residues in a right handed helix. Alternatively, a D-Cys containing metallopeptide allows creation of a topographic mimic for  $i$  and  $i + 4$  residues of a putative un-natural left helix.

It is also to be appreciated that while the natural and linear peptide and analogues are subjected to the confines of the Chou-Fasman type of rules (P.Y. Chou and G.D. Fasman: Prediction of protein structure, *Biochemistry* 13:222-245, 1974) that preclude inclusion of certain amino acid residues in particular types of secondary structures, the methods and constructs of this invention are completely independent of these rules. This invention allows incorporation of any natural or synthetic amino acid residues in the structure without Chou-Fasman rules limitations, and with virtually no other limitations.

It is to be appreciated here that while the structures shown in Figs. 1 - 7 have backbones that are very distinct from those in natural protein structures, the objective of this invention is to utilize the similarities in terms of positioning C- $\alpha$  carbon atoms, as well as C- $\beta$  carbon atoms in certain cases, of various amino acid residues in the same chemical spaces as in corresponding native protein structures, and further to derivatize these positions so as to achieve a chemical topology or surface similar to that in a natural bioactive structural motif. Utilizing these metallopeptide structures a biologically active molecule can therefore be identified that represents and defines the sites of folding or conformational constraints in a parent polypeptide, such as a peptide or protein. For a polypeptide with an unknown structure-function relationship, this information is generated by synthesizing a combination of all the metallopeptides corresponding to the parent polypeptide designed by inserting or substituting a Cys residue at some or all positions in the parent polypeptide. The structure of the biologically active metallopeptide in this series then elucidates the folding site in the polypeptide. This metallopeptide also provides information on key constrained amino acid residues, including but not limited to their relationship, including spatial relationship, to one another and their chirality. This information is then utilized to generate a molecular model, such as a computer-based molecular model, that defines a minimal structure pharmacophore model for further optimization. In the practice of this invention it is possible to utilize structural motifs thus identified by further modification of the defined topology to accentuate a desired biological effect, such as by substituting homologous amino acid side chains in place of naturally-occurring side chains in the parent polypeptide.

Examples of homologous side chains include, but are not limited to, substituting D-amino acid



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residues for an L-amino acid or utilizing homologues of an amino acid, such as for example the series phenylglycine, homophenylalanine, ring-substituted halogenated, and alkylated or arylated phenylalanines for a phenylalanine residue, diamino proionic acid, diamino butyric acid, ornithine, lysine and homoarginine for an arginine residue, and the like.

5 It may be seen that in the practice of the invention a free thiol or sulfhydryl (-SH) group of a residue is utilized for complexation of metal ions. Peptides and other organic molecules with free -SH groups, however, are easily oxidized in air and in solution, and can often form a disulfide-linked dimer. If more than one free -SH group is present in a molecule, oxidation may lead to a complex polymer. In addition, with more than one free -SH group when the metal ion is complexed to the peptide, it is possible to have metal ion complexation at more than one MCD in the peptide. This results in mixed species of metallopeptides, thereby complicating determination of the specific metallopeptide responsible for binding to a target of interest, as well as determination of the relevant secondary structure. Similarly, if a mixture of different peptides or organic molecules with free -SH groups are prepared, oxidation generally leads to a complex mixture of polymers of unknown composition. This is of serious concern in preparing libraries of metallopeptides or other organic molecules where one or more -SH group is intended for use in metal complexation.

10 In order to construct metallopeptides of this invention which incorporate an -SH group, and most particularly in order to construct libraries, it is desirable to employ S-protected derivatives. The S-protecting group is chosen such that (a) the synthesis of peptides with the S-protecting group is compatible with methods of solution and solid phase peptide synthesis, so that the S-protecting group is stable during synthetic procedures, and (b) the S-protecting group can be deprotected in situ, without cleavage from the resin in the case of solid phase synthesis, during the metal complexation step. An S-protecting group meeting the forgoing criteria is defined herein as an orthogonal S-protected group (OSPG). Many prior art methods meet at most only one of the two criteria specified above, and thus do not constitute an OSPG as defined herein.

20 Use of orthogonally S-protected thiol groups permits synthesis of metallo-compounds in a single vessel. A mixture of compounds, each compound containing an OSPG, is used for complexation with a metal ion, and it is only during metal ion complexation that the S-protected group is deprotected, and accordingly polymerization and cross-linking is avoided. This procedure thus provides homogenous libraries of metallopeptides.

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One OSPG meeting the criteria specified above, and which can be advantageously used in this invention, employs an S<sup>t</sup>Bu (S-thio-butyl or S-t-butyl) group to protect the -SH group. The S<sup>t</sup>Bu group is stable under both the acidic and basic conditions typically employed in peptide synthesis. Further, the S<sup>t</sup>Bu group may be cleaved by reduction using a suitable phosphine reagent, which  
5 reduction step may be employed immediately prior to, or in conjunction with, complexing of a metal ion to the peptide. Such OSPG cleavage does not cleave the peptide from the resin, or otherwise alter the structure of the peptide.

Another OSPG meeting the criteria specified above and suitable for this invention employs an S-Acm (S-acetamidomethyl) group to protect the -SH group. The Acm group is also stable under the  
10 acid and base conditions usually employed during peptide synthesis. The S-Acm group may be removed by treatment of S-Acm-protected peptide or peptide resin with mercury (II) acetate or silver (I) tetrafluoroborate, which liberates the thiol peptide in its mercury or silver ion-complexed state. If a mercury or silver ion metallopeptide is desired, the resulting metallopeptide may be kept in solution and employed in assays as described herein. Alternatively, free thiol-containing peptide can be  
15 recovered by treating the mercury or silver ion and thiol complexed salts with an excess of a thiol-containing reagent, such as beta-mercaptoethanol or dithiothreitol. The resulting peptide is then used for metal complexation to a metal such as Re or Tc. Alternatively, the mercury or silver ion and thiol complexed peptide may be directly treated with a metal ion complexing reagent, such as an Re complexing reagent, to form a desired metallopeptide, such as an Re metallopeptide.

20 Other examples of OSPGs for metallopeptides include 4-methoxytrityl (Mmt), 3-nitro-2-pyridinesulfonyl (Npys) and S-sulfonate (SO<sub>3</sub>H). Mmt is selectively removed upon treatment with 1% TFA in dichloromethane. Npys and S-sulfonate are selectively removed by treatment with a thiol-containing reagent such as beta-mercaptoethanol or dithiothreitol or a phosphine reagent such as tributyl phosphine. The Npys group (R.G. Simmonds RG et al: *Int J Peptide Protein Res*,  
25 43:363,1994) is compatible with Boc chemistry for peptide synthesis and the S-sulfonate (Maugras I et al: *Int J Peptide Protein Res*, 45:152, 1995) is compatible with both Fmoc and Boc chemistries. Similar OSPGs derived from homologous series of S-alkyl, or S-aryl, or S-aralkyl may also be used in this invention. A primary characterization of the OSPG is that its use results in the formation of a disulfide (S-S) bond utilizing one sulfur atom each from the thiol-containing amino acid and the

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protecting group. In addition, the resulting disulfide bond is cleavable by the use of any of a variety of disulfide cleaving agents, including but not limited to phosphine- and thiol-containing reagents.

The method employing S<sup>t</sup>Bu protected -SH groups, or other OSPGs, may be employed for the generation of either solid phase or soluble libraries. For solid phase libraries, peptides may be synthesized by use of conventional Fmoc chemistry. In the case of conventional Fmoc chemistry, Fmoc-L-Cys-(S<sup>t</sup>Bu) is coupled to an appropriate resin, via one or more intermediate amino acid residues, and additional amino acid residues are thereafter coupled to the L-Cys-(S<sup>t</sup>Bu) residue. S<sup>t</sup>Bu may be employed with either L- or D-Cys, and any of a variety of other amino acid residues, including designer or unnatural amino acid residues and mimics thereof, characterized by an -SH group available for complexation to a metal ion, including, but not limited to, 3-mercapto phenylalanine and other related 3-mercapto amino acid residues such as 3-mercapto valine (penicillamine), all of the foregoing of which constitute an N<sub>1</sub>S<sub>1</sub> residue. In all these cases, S- protection can be by S-Bu<sup>t</sup>, S-Acm, Mmt, Npys, S-sulfonate and related groups, as described above.

The complexation of metal ions to the peptides, including peptides in a library, and specifically to the MCD of peptides, is achieved by mixing the peptides with the metal ion. This is conveniently done in solution, with the solution including an appropriate buffer. In one approach, the metal ion is, when mixed with the peptide or peptidomimetic constituents, already in the oxidation state most preferred for complexing to the MCD. Some metal ions are complexed in their most stable oxidation state, such as calcium (II), potassium (I), indium (III), manganese (II), copper (II), zinc (II) and other metals. In other instances, the metal must be reduced to a lower oxidation state in order to be complexed to the MCD. This is true of ferrous, ferric, stannous, stannic, technetiumoxo[V], pertechnetate, rheniumoxo[V], perrhenate and other similar metal ions. Reduction may be performed prior to mixing with the sequences, simultaneously with mixing with the sequences, or subsequent to mixing with the sequences. Any means of reduction of metal ions to the desired oxidation state known to the art may be employed.

Re and Tc are preferred metal ions to employ, particularly in that the resulting metallopeptides may be purified and removed from solution, such as by lyophilization, and remain stable. Other metallopeptides, as for example metallopeptides utilizing Zn, Cu, Ni, Co, Fe and Mn are stable in solution, but are prone to oxidation and loss of the metal ion if removed from solution. Thus these metallopeptides must be kept in solution, and optimally at the appropriate pH and with appropriate

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buffers, at all times, including during conduct of assays and other tests. This imparts some limitations on the utility of these metal ions; however, metallopeptides utilizing metal ions other than Re or Tc may be employed as discussed herein.

For tetradentate coordination with a metal ion, rhenium or technetium are preferred ions.

- 5 Because of its ready availability and the stability of the coordination complex, Re is a particularly preferred metal ion. Solid phase resin bound peptide or peptidomimetic sequences may be labeled with rhenium ion by treatment with the rhenium transfer agent  $\text{ReOCl}_3(\text{PPh}_3)_2$  in the presence of a base, such as 1,8-Diazabicyclo[5,4,0] undec-7-ene (DBU). The sequences may then be cleaved from the resin. Peptide or peptidomimetic sequences in solution may similarly be labeled by
- 10 treatment with the rhenium transfer agent  $\text{ReOCl}_3(\text{PPh}_3)_2$  in the presence of a base, such as triethylamine, diisopropylethylamine, N-methylmorpholine or DBU. Metal complexation in the presence of DBU as a base can conveniently be accomplished at ambient room temperature.

- In an alternative method of metal complexation a mild base, such as sodium acetate, can be used. In this case the thiol-containing sequence, either in solution or bound to solid phase, is taken
- 15 in a suitable solvent, such as dimethylformamide (DMF), dichloromethane (DCM), N-methylpyrrolidinone (NMP), methanol (MeOH) or a mixture thereof, and heated to 60-70° C with the rhenium transfer agent  $\text{ReOCl}_3(\text{PPh}_3)_2$  in the presence of sodium acetate for 15 minutes. Similarly, other bases such as triethylamine, ammonium hydroxide and so on, may be employed. According to this invention, MeOH is a preferred choice of solvent for rhenium complexation in the case of S-
- 20 deprotected peptides in solution. The solvent choice for S-deprotected peptides still attached to the solid phase is guided mainly by considerations of superior solvation (swelling) of the solid phase. DMF and NMP may be employed. Various mixtures of these solvents, also in combination with MeOH, and DCM,  $\text{CHCl}_3$  and so on, may also be employed to yield optimized complexation results.

- In one embodiment of this invention, an  $\text{S}^t\text{Bu}$  protected peptide is treated in situ with rhenium
- 25 transfer agent in the presence of DBU and tributylphosphine to effect S-deprotection and rhenium complexation in one vessel. Alternately, complexing of rhenium to the  $\text{S}^t\text{Bu}$  protected peptide in the presence of rhenium perrhenate may be accomplished by treatment with  $\text{Sn}(\text{II})\text{Cl}_2$ . This reagent effects S-deprotection as well as conversion of the  $\text{ReO}_4$  state to an  $\text{ReO}$  state in situ to thereby effect complexation of the rhenium to the S-deprotected peptide. A preferred procedure in this
- 30 invention is the use of  $\text{S-Bu}^t$  protected peptide with S-deprotection by treatment with

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tributylphosphine, and metal complexation of the resulting peptide utilizing  $\text{ReOCl}_3(\text{PPh}_3)_2$  in the presence of DBU at room temperature.

It is possible and contemplated to prepare libraries of peptides of this invention, and to then complex the resulting peptides to a metal ion, such as rhenium, resulting in a metallopeptide. Such a library may be a solid phase library, or may be a solution phase library.

A peptide library is first assembled based on the parent polypeptide, as described above, by well-known methods of peptide synthesis. Both solid-phase and soluble libraries can be obtained in this manner. The entire library is then reacted with an appropriate metal-complexing agent to obtain the corresponding metal-coordinated library, comprising a similar class of predetermined structures.

For example, to complex a peptide library with rheniumoxo metal ion, the peptide library can be treated with  $\text{Re}(\text{O})\text{Cl}_3(\text{PPh}_3)_2$  in the presence of sodium acetate. This procedure results in quantitative complexation of  $\text{ReO}$  with the peptide. In order to complex Zn, Ni, Co, Mn, Fe or Cu ions, the peptide library is treated with chloride or other suitable salts of these metal ions to yield the library of corresponding metal ions. Essentially, a variety of metal ions can be used to construct different metallopeptide libraries. One limiting factor in selection of the appropriate metal ion is the relative stability of a particular metal-peptide complex, related in large part to the metal-peptide complex binding constant or constants. It is well known in the art that some metal-peptide constructs are stable only within specified pH or other special conditions, or are easily oxidized in air. Other peptide-metal ion complexes, such as those with  $\text{ReO}$ , are stable in pure form and can be isolated and stored under normal storage conditions for a long period of time.

In a preferred embodiment a solid-phase methodology is employed for the synthesis of metallopeptides, in which the metal ion complexation is also achieved while the peptide is on the solid phase. Using Fmoc chemistry a linear peptide is fully assembled on rink amide resin using a  $\text{S}^t\text{Bu}$  protected Cys derivative. Following synthesis of the peptide, the  $\text{S}^t\text{Bu}$  group is removed by treatment with  $\text{Bu}_3\text{P}$  in DMF. The resulting free -SH containing peptide-resin is treated with the rhenium transfer reagent  $\text{ReO}[\text{V}]\text{Cl}_3(\text{PPh}_3)_2$  in presence of DBU as base. Complete metal-ion complexation is achieved within 2 hours at room temperature. The resulting metallopeptide resin is washed, dried and then treated with TFA to cleave the metallopeptide from the resin and remove all side chain protecting groups. The metallopeptide is purified by HPLC and characterized by mass spectrometry and amino acid analysis.

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The invention is further illustrated by the following non-limiting example:

### **Example 1**

The amino terminal fragment (ATF) of urokinase-type tissue plasminogen activator (uPA) protein is sufficient for binding to the uPA receptor. In particular, the binding capability has been demonstrated to be within the omega loop composed of the 21-30 amino acid sequence of ATF that is encased within a Cys-Cys disulfide bridge. An N- and C-terminally capped 11-amino acid peptide corresponding to this omega loop sequence was selected for making a series of Re-complexed metallopeptides to determine the structure and location of the biologically relevant reversed turn structure within this sequence. The parent polypeptide, here a parent peptide, with the sequence Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:2) was subjected to a series of systematic Cys insertions starting after the 2 position and to the n+1 position, where n was the number of residues in the parent peptide. A series of ten peptides were synthesized by standard methods of solid-phase peptide synthesis. The -SH group of Cys was protected with an orthogonal S<sup>t</sup>-Bu group. After the complete assembly of each individual peptide on resin the S<sup>t</sup>-Bu group was removed by treatment with tributylphosphine and the peptide resin then treated with the Re-oxo transfer agent Re(O)Cl<sub>3</sub>(PPh<sub>3</sub>)<sub>2</sub> in the presence of DBU to form a metallopeptide. The peptide resin was then treated with TFA to cleave the resulting metallopeptide from the resin. The metallopeptides were purified by high precision liquid chromatography (HPLC) and assayed in receptor-binding assay using U937 cells and ATF as the competitive receptor binding ligand. The data presented in **Table 1** shows that the peptide Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Cys-Trp-NH<sub>2</sub> (SEQ ID NO:3) bound to a rhenium ion to form a metallopeptide was the most potent of all these molecules, and signified location of BD around the Ile-His-Cys-Trp fragment of the peptide. Other compounds in the table presented turn structures that were not associated with the pharmacophore involved with the uPA receptor binding. This series of ten systematically synthesized molecules was therefore sufficient to delineate the location of the turn segment in this peptide fragment. In **Table 1**, the assignments of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are as shown in the template of **Fig. 11**.

<b>Table 1</b> <b>uPA Receptor Binding Data Showing</b> <b>Percentage Inhibition Of Binding of Metallopeptides</b> <b>with the Defined Amino Sequence</b>					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>Cys</b>	<b>R<sub>4</sub></b>	<b>% Inhibition at 1 <math>\mu</math>M</b>
Ac-	Val	Ser-	Cys-	Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-NH <sub>2</sub> (SEQ ID NO:4)	0.0
Ac-Val-	Ser-	Asn	Cys-	Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-NH <sub>2</sub> (SEQ ID NO:5)	0.0
Ac-Val-Ser-	Asn-	Lys-	Cys-	Tyr-Phe-Ser-Asn-Ile-His-Trp-NH <sub>2</sub> (SEQ ID NO:6)	13.0
Ac-Val-Ser-Asn-	Lys-	Tyr-	Cys-	Phe-Ser-Asn-Ile-His-Trp-NH <sub>2</sub> (SEQ ID NO:7)	18.0
Ac-Val-Ser-Asn-Lys-	Tyr-	Phe	Cys-	Ser-Asn-Ile-His-Trp-NH <sub>2</sub> (SEQ ID NO:8)	0.0
Ac-Val-Ser-Asn-Lys-Tyr-	Phe-	Ser-	Cys-	Asn-Ile-His-Trp-NH <sub>2</sub> (SEQ ID NO:9)	35.0
Ac-Val-Ser-Asn-Lys-Tyr-Phe-	Ser-	Asn-	Cys-	Ile-His-Trp-NH <sub>2</sub> (SEQ ID NO:10)	32.0
Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-	Asn-	Ile-	Cys-	His-Trp-NH <sub>2</sub> (SEQ ID NO:11)	0.0
Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-	Ile-	His-	Cys-	Trp-NH <sub>2</sub> (SEQ ID NO:3)	106.0
Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-	His-	Trp-	Cys-NH <sub>2</sub>	(SEQ ID NO:12)	0.0

### **Example 2**

Ac-Nle-Ala-His-D-Phe-Arg-Trp-NH<sub>2</sub> is a known receptor-binding sequence for melanotropin receptors. The K<sub>i</sub> values for binding to MCR-1 and -4 were measured to be 0.1  $\mu$ M and 2  $\mu$ M respectively. A series of metallopeptides based on this sequence were synthesized by inserting a Cys residue after the 2 position and through the n+1 position and complexing the resulting Cys-containing peptide with an Re-oxo metal ion core as described Example 1. The resulting metallopeptides were screened for inhibiting the binding of <sup>125</sup>I-NDP-alpha-MSH radioligand using B-16 mouse melanoma cells for MCR-1 and cloned human MCR-2, -3 and -4 receptor transfected 293 cells.

The competitive inhibition binding assay was conducted using membranes prepared from hMC3-R, hMC4-R, hMC5-R, and B-16 mouse melanoma cells (containing MC1-R) using 0.4 nM <sup>125</sup>I-

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NDP-alpha-MSH (New England Nuclear, Boston, MA, USA) in 50 mM HEPES buffer containing 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, and 5 mM KCl, at pH 7.2. The assay tube also contained a chosen concentration of the test peptide of this invention, complexed to a rhenium metal ion as indicated, for determining its efficacy in inhibiting the binding of <sup>125</sup>I-NDP-alpha-MSH to its receptor. Non-specific binding was measured by complete inhibition of binding of <sup>125</sup>I-NDP-alpha-MSH in the assay with the presence of 1 μM alpha-MSH. Incubation was for 90 minutes at room temperature, after which the assay mixture was filtered and the membranes washed three times with ice cold buffer. The filter was dried and counted in a gamma counter for remaining radioactivity bound to the membranes. 100% specific binding was defined as the difference in radioactivity (cpm) bound to cell membranes in the absence and presence of 1 μM alpha-MSH. The cpm obtained in presence of test compounds were normalized with respect to 100% specific binding to determine the percent inhibition of <sup>125</sup>I-NDP-alpha MSH binding. Each assay was conducted in triplicate and the actual mean values are described in **Table 2**.

The data is presented in **Table 2**. It was evident that the metallopeptide Ac-Nle-Ala-His-D-Phe-Arg-Cys-Trp-NH<sub>2</sub> presented a conformationally constrained structure obtained by the complexation of the rheniumoxo metal ion, which structure was a BD specific for the MCR-1 receptor but not the MCR-4 receptor. The locus of this structural motif was included in the D-Phe-Arg-Cys-Trp sequence. This turn motif therefore led to the development of a potent MCR-1 specific ligand. The constrained structural motif with the D-Phe-Arg-Trp-Cys sequence locus, Ac-Nle-Ala-His-D-Phe-Arg-Trp-Cys-NH<sub>2</sub>, presented a pharmacophore for binding both the MCR-1 and MCR-4 receptors. In **Table 1**, the assignments of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are as shown in the template of **Fig. 11**.



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<b>Table 2</b> <b>Melanocortin Receptor Binding Data Showing</b> <b>Percentage Inhibition Of Binding of Metallopeptides</b> <b>with the Defined Amino Sequence</b>								
R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Cys	R <sub>4</sub>	% Inhibition at 1 $\mu$ M			
					MC-1	MC-3	MC-4	MC-5
Ac-	Nle-	Ala-	Cys-	His-D-Phe-Arg-Trp-NH <sub>2</sub>	96	47	71	64
Ac-Nle-	Ala-	His-	Cys-	D-Phe-Arg-Trp-NH <sub>2</sub>	84	9	75	58
Ac-Nle-Ala-	His-	D-Phe-	Cys-	Arg-Trp-NH <sub>2</sub>	93	15	66	57
Ac-Nle-Ala-His-	D-Phe-	Arg-	Cys-	Trp-NH <sub>2</sub>	96	0	17	0
Ac-Nle-Ala-His-D-Phe-	Arg-	Trp-	Cys-	NH <sub>2</sub>	91	70	98	93

**Example 3**

*Alzheimer's and Prion Diseases.* Alzheimer's and prion diseases, such as Creutzfeldt-Jakob disease and related prion-driven diseases, are disorders of protein conformation. These are neurodegenerative diseases that lead to dementia. In most cases the disease is due to a set of conformational changes in the respective disease associated protein, amyloid- $\beta$  in the case of Alzheimer's disease, and glycoprotein PrP<sup>Sc</sup> in the case of prion disease, which results in high level of beta-sheet structural motif. It has been shown that a peptide related to a specific sequence of respective protein with the additional ability to destabilize the formation of the beta sheet and capable of binding to the disease state protein conformer may serve as a useful therapeutic to halt progression of the disease, and may even effect its reversal. Other researchers have developed a series of linear peptides that have shown specific binding to the disease state protein and show promise of their therapeutic potential. See, for example, Soto C: Plaque busters: Strategies to inhibit amyloid formation in Alzheimer's Disease. *Mol. Medicine Today*, 5: 343-350 (1999); Soto C. et al.: Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: Implications for Alzheimer's therapy. *Nature Medicine*, 4: 822-826 (1998); Soto C: Alzheimer's and prion disease as disorders of protein conformation: Implications for the design of novel therapeutic approaches. *J. Mol. Med.*, 77: 412-418 (1999); and Soto C et al.: Reversion of prion protein conformational changes by synthetic beta-sheet breaker peptides. *The Lancet*, 355: 192-197 (2000).

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The metallopeptides of this invention may be used to conformationally restrict a portion of the parent polypeptide based on the co-ordination of Re metal ion to at least a portion of the amino acid sequence thereof. The resulting metallopeptide is proteolytically stable and is generally relatively more hydrophobic than the corresponding parent peptide. A base metallopeptide template may also be decorated with appropriate side chain functionalities to generate topographies that mimic the bioactive topography of a natural peptide, for example, peptides related to Alzheimer's and prion disease.

*Representative Alzheimer's Disease Peptides of the Invention.* The 17-20 hydrophobic region peptide (LVFF) serves in part as a template for developing specific beta-sheet breaker peptides. The linear peptide sequences of Table 3 are used as a starting template for rational design of peptide sequences which, when bound to a metal ion such as rhenium, form a metallopeptide.

**Table 3**

**Amyloid Beta-Protein Related Peptides for Treatment of AD**

His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val (SEQ ID NO:13)

Ac-Leu-Ala-Phe-Phe-Asp-NH<sub>2</sub> (SEQ ID NO:14)

Ac-Leu-Pro-Phe-Phe-Asp-NH<sub>2</sub> (SEQ ID NO:15)

The parent polypeptides or peptides described in Table 3 can be employed as the template basis for synthesizing a series of metallopeptides, using the methods and constructs of this invention, with either L-Cys or D-Cys. In the practice of this invention, an N<sub>1</sub>S<sub>1</sub> residue is employed, such as cysteine, which may be either L-Cys or D-Cys. Peptides are constructed using standard peptide synthesis techniques, in which the cysteine is inserted at selected points. The -SH group of Cys may be protected using an orthogonal protecting agent as set forth above. The resulting Cys-containing peptides are then deprotected, and subsequently complexed with a rhenium ion, forming a metallopeptide, using a suitable pre-formed metal-oxo transfer agent such as Re(O)Cl<sub>3</sub>(PPh<sub>3</sub>)<sub>2</sub>. Through use of competitive inhibition assays, the binding of each of the resulting metallopeptides is compared against the parent peptide, and those with enhanced or increased binding are identified.

Utilizing this approach, a series of initial and precursor metallopeptides are defined as set forth in Table 4.

**Table 4****Precursor Metallopeptides for AD**R<sub>1</sub>-His-Gln-Lys-Leu-Aaa-Phe-Phe-Ala-Glu-Asp-Val-Cys-R<sub>2</sub> (SEQ ID NO:16)5 R<sub>1</sub>-His-Gln-Lys-Leu-Aaa-Phe-Phe-Ala-Glu-Asp-Cys-Val-R<sub>2</sub> (SEQ ID NO:17)R<sub>1</sub>-His-Gln-Lys-Leu-Aaa-Phe-Phe-Ala-Glu-Cys-Asp-Val-R<sub>2</sub> (SEQ ID NO:18)R<sub>1</sub>-His-Gln-Lys-Leu-Aaa-Phe-Phe-Ala-Cys-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:19)R<sub>1</sub>-His-Gln-Lys-Leu-Aaa-Phe-Phe-Cys-Ala-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:20)R<sub>1</sub>-His-Gln-Lys-Leu-Bbb-Phe-Phe-Cys-Ala-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:21)10 R<sub>1</sub>-His-Gln-Lys-Leu-Bbb-Phe-Cys-Phe-Ala-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:22)R<sub>1</sub>-His-Gln-Lys-Leu-Bbb-Cys-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:23)R<sub>1</sub>-His-Gln-Lys-Leu-Cys-Aaa-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:24)R<sub>1</sub>-His-Gln-Lys-Cys-Leu-Aaa-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:25)R<sub>1</sub>-His-Gln-Cys-Lys-Leu-Aaa-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub> (SEQ ID NO:26)

15 Where:

R<sub>1</sub> is H (N-terminus is free amino group) or Ac (Acetyl group at N-terminus);R<sub>2</sub> is OH (free carboxylate at C-terminus) or NH<sub>2</sub> (C-terminal is amide group);

Aaa is Val, Pro, Gly or Ala;

Bbb is Val, Gly or Ala;

20 Cys is either L-Cys or D-Cys; and

the three amino acid residues preceding Cys and the one amino acid immediately following Cys are either L-amino acid residues or D-amino acid residues, or any combination thereof.

The following series of peptides are derived from the series of peptides of Table 4. In each of these series the length of peptide is shortened successively either from the N- or the C-termini, or both. In the following series (Table 5 through Table 14), R<sub>1</sub>, R<sub>2</sub>, Aaa, Bbb and Cys are as defined, with the three amino acid residues preceding Cys and the one amino acid immediately following Cys either L-amino acid residues or D-amino acid residues, or any combination thereof.

**Table 5**R<sub>3</sub>-Asp-Val-Cys-R<sub>2</sub>30 where R<sub>3</sub> is R<sub>1</sub>-Gln-Lys-Leu-Aaa-Phe-Phe-Ala-Glu, (SEQ ID NO:27)

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R<sub>1</sub>-Lys-Leu-Aaa-Phe-Phe-Ala-Glu, (SEQ ID NO:28)R<sub>1</sub>-Leu-Aaa-Phe-Phe-Ala-Glu, (SEQ ID NO:29)R<sub>1</sub>-Aaa-Phe-Phe-Ala-Glu, (SEQ ID NO:30)R<sub>1</sub>-Phe-Phe-Ala-Glu, (SEQ ID NO:31)5 R<sub>1</sub>-Phe-Ala-Glu,R<sub>1</sub>-Ala-Glu,R<sub>1</sub>-Glu, orR<sub>1</sub>.

10

**Table 6**R<sub>4</sub>-Glu-Asp-Cys-R<sub>5</sub>where R<sub>4</sub> is R<sub>1</sub>-His-Gln-Lys-Leu-Aaa-Phe-Phe-Ala, (SEQ ID NO:32)R<sub>1</sub>-Gln-Lys-Leu-Aaa-Phe-Phe-Ala, (SEQ ID NO:33)R<sub>1</sub>-Lys-Leu-Aaa-Phe-Phe-Ala, (SEQ ID NO:34)

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R<sub>1</sub>-Leu-Aaa-Phe-Phe-Ala, (SEQ ID NO:35)R<sub>1</sub>-Aaa-Phe-Phe-Ala,R<sub>1</sub>-Phe-Phe-Ala,R<sub>1</sub>-Phe-Ala,R<sub>1</sub>-Ala, or

20

R<sub>1</sub>;and R<sub>5</sub> is Val-R<sub>2</sub>, orR<sub>2</sub>.**Table 7**

25

R<sub>6</sub>-Ala-Glu-Cys-R<sub>7</sub>where R<sub>6</sub> is R<sub>1</sub>-His-Gln-Lys-Leu-Aaa-Phe-Phe, (SEQ ID NO:36)R<sub>1</sub>-Gln-Lys-Leu-Aaa-Phe-Phe, (SEQ ID NO:37)R<sub>1</sub>-Lys-Leu-Aaa-Phe-Phe, (SEQ ID NO:38)R<sub>1</sub>-Leu-Aaa-Phe-Phe,

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R<sub>1</sub>-Aaa-Phe-Phe,

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$R_1$ -Phe-Phe,  
 $R_1$ -Phe, or  
 $R_1$ ;  
 and  $R_7$  is  
 Asp-Val- $R_2$ ,  
 Asp- $R_2$ , or  
 $R_2$ .

5

Table 8

$R_8$ -Phe-Ala-Cys- $R_9$   
 where  $R_8$  is  
 $R_1$ -His-Gln-Lys-Leu-Aaa-Phe, (SEQ ID NO:39)  
 $R_1$ -Gln-Lys-Leu-Aaa-Phe, (SEQ ID NO:40)  
 $R_1$ -Lys-Leu-Aaa-Phe,  
 $R_1$ -Leu-Aaa-Phe,  
 $R_1$ -Aaa-Phe,  
 $R_1$ -Phe, or  
 $R_1$ ;  
 and  $R_9$  is  
 Glu-Asp-Val- $R_2$ ,  
 Glu-Asp- $R_2$ ,  
 Glu- $R_2$ , or  
 $R_2$ .

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15

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Table 9

$R_9$ -Phe-Phe-Cys- $R_{10}$   
 where  $R_9$  is  
 $R_1$ -His-Gln-Lys-Leu-Aaa, (SEQ ID NO:41)  
 $R_1$ -Gln-Lys-Leu-Aaa,  
 $R_1$ -Lys-Leu-Aaa,  
 $R_1$ -Leu-Aaa,  
 $R_1$ -Aaa,  
 $R_1$ ,  
 $R_1$ -His-Gln-Lys-Leu-Bbb, (SEQ ID NO:42)

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R<sub>1</sub>-Gln-Lys-Leu-Bbb,R<sub>1</sub>-Lys-Leu-Bbb,R<sub>1</sub>-Leu-Bbb, orR<sub>1</sub>-Bbb;5           and R<sub>10</sub> is   Ala-Glu-Asp-Val-R<sub>2</sub>, (SEQ ID NO:43)Ala-Glu-Asp-R<sub>2</sub>,Ala-Glu-R<sub>2</sub>,Glu-R<sub>2</sub>, orR<sub>2</sub>.

10

**Table 10**R<sub>11</sub>-Bbb-Phe-Cys-R<sub>12</sub>where R<sub>11</sub> is   R<sub>1</sub>-His-Gln-Lys-Leu, (SEQ ID NO:44)R<sub>1</sub>-Gln-Lys-Leu,

15

R<sub>1</sub>-Lys-Leu,R<sub>1</sub>-Leu, orR<sub>1</sub>;and R<sub>12</sub> is   Phe-Ala-Glu-Asp-Val-R<sub>2</sub>, (SEQ ID NO:45)Phe-Ala-Glu-Asp-R<sub>2</sub>, (SEQ ID NO:46)

20

Phe-Ala-Glu-R<sub>2</sub>,Phe-Glu-R<sub>2</sub>,Phe-R<sub>2</sub>, orR<sub>2</sub>.

25

**Table 11**R<sub>13</sub>-Leu-Bbb-Cys-R<sub>14</sub>where R<sub>13</sub> is   R<sub>1</sub>-His-Gln-Lys,R<sub>1</sub>-Gln-Lys,R<sub>1</sub>-Lys, or

30

R<sub>1</sub>;

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and R<sub>14</sub> is Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub>, (SEQ ID NO:47)  
 Phe-Phe-Ala-Glu-Asp-R<sub>2</sub>, (SEQ ID NO:48)  
 Phe-Phe-Ala-Glu-R<sub>2</sub>, (SEQ ID NO:49)  
 Phe-Phe-Glu-R<sub>2</sub>,  
 5 Phe-Phe-R<sub>2</sub>,  
 Phe-R<sub>2</sub>, or  
 R<sub>2</sub>.

**Table 12**

10 R<sub>15</sub>-Lys-Leu-Cys-R<sub>16</sub>  
 where R<sub>15</sub> is R<sub>1</sub>-His-Gln,  
 R<sub>1</sub>-Gln, or  
 R<sub>1</sub>;  
 and R<sub>16</sub> is  
 15 Aaa-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub>, (SEQ ID NO:50)  
 Aaa-Phe-Phe-Ala-Glu-Asp-R<sub>2</sub>, (SEQ ID NO:51)  
 Aaa-Phe-Phe-Ala-Glu-R<sub>2</sub>, (SEQ ID NO:52)  
 Aaa-Phe-Phe-Glu-R<sub>2</sub>,  
 Aaa-Phe-Phe-R<sub>2</sub>,  
 Aaa-Phe-R<sub>2</sub>,  
 20 Aaa-R<sub>2</sub>,  
 R<sub>2</sub>,  
 Bbb-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub>, (SEQ ID NO:53)  
 Bbb-Phe-Phe-Ala-Glu-Asp-R<sub>2</sub>, (SEQ ID NO:54)  
 Bbb-Phe-Phe-Ala-Glu-R<sub>2</sub>, (SEQ ID NO:55)  
 25 Bbb-Phe-Phe-Glu-R<sub>2</sub>,  
 Bbb-Phe-Phe-R<sub>2</sub>,  
 Bbb-Phe-R<sub>2</sub>, or  
 Bbb-R<sub>2</sub>.

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**Table 13**R<sub>17</sub>-His-Gln-Lys-Cys-R<sub>18</sub> (SEQ ID NO:56)where R<sub>17</sub> is R<sub>1</sub>-His orR<sub>1</sub>;5 and R<sub>18</sub> is Leu-Aaa-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub>, (SEQ ID NO:57)Leu-Aaa-Phe-Phe-Ala-Glu-Asp-R<sub>2</sub>, (SEQ ID NO:58)Leu-Aaa-Phe-Phe-Ala-Glu-R<sub>2</sub>, (SEQ ID NO:59)Leu-Aaa-Phe-Phe-Glu-R<sub>2</sub>, (SEQ ID NO:60)Leu-Aaa-Phe-Phe-R<sub>2</sub>,10 Leu-Aaa-Phe-R<sub>2</sub>,Leu-Aaa-R<sub>2</sub>,Leu-R<sub>2</sub>, orR<sub>2</sub>.

15

**Table 14**R<sub>1</sub>-His-Gln-Cys-R<sub>19</sub>where R<sub>19</sub> is Lys-Leu-Aaa-Phe-Phe-Ala-Glu-Asp-Val-R<sub>2</sub>, (SEQ ID NO:61)Lys-Leu-Aaa-Phe-Phe-Ala-Glu-Asp-R<sub>2</sub>, (SEQ ID NO:62)Lys-Leu-Aaa-Phe-Phe-Ala-Glu-R<sub>2</sub>, (SEQ ID NO:63)20 Lys-Leu-Aaa-Phe-Phe-Glu-R<sub>2</sub>, (SEQ ID NO:64)Lys-Leu-Aaa-Phe-Phe-R<sub>2</sub>, (SEQ ID NO:65)Lys-Leu-Aaa-Phe-R<sub>2</sub>,Lys-Leu-Aaa-R<sub>2</sub>,Lys-Leu-R<sub>2</sub>,25 Lys-R<sub>2</sub>, orR<sub>2</sub>.

*Representative Prion Disease Peptides of the Invention.* In another embodiment of this invention, peptides are provided for use in treatment of prion disease, including but not limited to Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease and related prion driven disorders. The linear

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peptide sequence of **Table 15** is used as a parent peptide for the rational design of peptide sequences which, when bound to a metal ion such as rhenium, form a metallopeptide.

**Table 15**

5 Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val (SEQ ID NO:66)

The parent peptide described in **Table 15** can be employed as the template basis for synthesizing a series of metallopeptides, using the methods and constructs of this invention, with either L-Cys or D-Cys. In the practice of this invention, an N<sub>1</sub>S<sub>1</sub> residue is employed, such as

10 cysteine, which may be either L-Cys or D-Cys. Peptides are constructed using standard peptide synthesis techniques, in which the cysteine is inserted at selected points. The -SH group of Cys may be protected using an orthogonal protecting agent as set forth above. The resulting Cys-containing peptides are then deprotected, and subsequently complexed with a rhenium ion, forming a metallopeptide, using a suitable pre-formed metal-oxo transfer agent such as Re(O)Cl<sub>3</sub>(PPh<sub>3</sub>)<sub>2</sub>.

15 Through use of competitive inhibition assays, the binding of each of the resulting metallopeptides is compared against the parent peptide, and those with enhanced or increased binding are identified.

Utilizing this approach, a series of precursor molecules are defined as set forth in **Table 16**.

**Table 16****Precursor Metallopeptides for Prion Disease**

20 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Aaa-Val-Cys-S<sub>2</sub> (SEQ ID NO:67)  
 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Aaa-Cys-Val-S<sub>2</sub> (SEQ ID NO:68)  
 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Bbb-Ala-Val-Cys-Bbb-Val-S<sub>2</sub> (SEQ ID NO:69)  
 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Aaa-Ala-Cys-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:70)  
 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Aaa-Cys-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:71)  
 25 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Bbb-Ala-Gly-Cys-Bbb-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:72)  
 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Aaa-Ala-Cys-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:73)  
 S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Aaa-Cys-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:74)  
 S<sub>1</sub>-Asp-Ala-Bbb-Ala-Ala-Cys-Bbb-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:75)  
 S<sub>1</sub>-Asp-Ala-Aaa-Ala-Cys-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:76)  
 30 S<sub>1</sub>-Asp-Ala-Aaa-Cys-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:77)

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S<sub>1</sub>-Asp-Ala-Cys-Bbb-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub> (SEQ ID NO:78)

where:

S<sub>1</sub> is H (N-terminus is free amino group) or Ac (Acetyl group at N-terminus);

S<sub>2</sub> is OH (free carboxylate at C-terminus) or NH<sub>2</sub> (C-terminal is amide group);

5       Aaa is Gly or Ala;

Bbb is Pro, Gly or Ala; and

the three amino acid residues preceding Cys and the one amino acid immediately following Cys are either L-amino acid residues or D-amino acid residues, or any combination thereof.

10       The following series of peptides are derived from the series of peptides of **Table 16**. In each of these series the length of peptide is shortened successively either from the N- or the C-termini, or both. In the following series (**Table 17** through **Table 28**), S<sub>1</sub>, S<sub>2</sub>, Aaa, Bbb and Cys are as defined, with the three amino acid residues preceding Cys and the one amino acid immediately following Cys either L-amino acid residues or D-amino acid residues, or any combination thereof.

**Table 17**

15       S<sub>3</sub>-Aaa-Val-Cys-S<sub>2</sub>

where S<sub>3</sub> is S<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val, (SEQ ID NO:79)

S<sub>1</sub>-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val, (SEQ ID NO:80)

S<sub>1</sub>-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val, (SEQ ID NO:81)

S<sub>1</sub>-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val, (SEQ ID NO:82)

20       S<sub>1</sub>-Ala-Pro-Ala-Gly-Pro-Ala-Val, (SEQ ID NO:83)

S<sub>1</sub>-Pro-Ala-Gly-Pro-Ala-Val, (SEQ ID NO:84)

S<sub>1</sub>-Ala-Gly-Pro-Ala-Val, (SEQ ID NO:85)

S<sub>1</sub>-Gly-Pro-Ala-Val, (SEQ ID NO:86)

S<sub>1</sub>-Pro-Ala-Val,

25       S<sub>1</sub>-Ala-Val,

S<sub>1</sub>-Val, or

S<sub>1</sub>.

**Table 18**

30       S<sub>4</sub>-Val-Aaa-Cys-S<sub>5</sub>

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where  $S_4$  is  $S_1$ -Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala, (SEQ ID NO:87)  
 $S_1$ -Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala, (SEQ ID NO:88)  
 $S_1$ -Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala, (SEQ ID NO:89)  
 $S_1$ -Ala-Ala-Pro-Ala-Gly-Pro-Ala, (SEQ ID NO:90)  
 $S_1$ -Ala-Pro-Ala-Gly-Pro-Ala, (SEQ ID NO:91)  
 $S_1$ -Pro-Ala-Gly-Pro-Ala, (SEQ ID NO:92)  
 $S_1$ -Ala-Gly-Pro-Ala, (SEQ ID NO:93)  
 $S_1$ -Gly-Pro-Ala,  
 $S_1$ -Pro-Ala,  
 $S_1$ -Ala, or  
 $S_1$ ;  
 and  $S_5$  is Val- $S_2$  or  
 $S_2$ .

Table 19

$S_6$ -Ala-Val-Cys- $S_7$   
 where  $S_6$  is  $S_1$ -Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Bbb, (SEQ ID NO:94)  
 $S_1$ -Ala-Pro-Ala-Ala-Pro-Ala-Gly-Bbb, (SEQ ID NO:95)  
 $S_1$ -Pro-Ala-Ala-Pro-Ala-Gly-Bbb, (SEQ ID NO:96)  
 $S_1$ -Ala-Ala-Pro-Ala-Gly-Bbb, (SEQ ID NO:97)  
 $S_1$ -Ala-Pro-Ala-Gly-Bbb, (SEQ ID NO:98)  
 $S_1$ -Pro-Ala-Gly-Bbb,  
 $S_1$ -Ala-Gly-Bbb,  
 $S_1$ -Gly-Bbb,  
 $S_1$ -Bbb, or  
 $S_1$ ;  
 and  $S_7$  is Bbb-Val- $S_2$ ,  
 Val- $S_2$ , or  
 $S_2$ .

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**Table 20**

	$S_8$ -Aaa-Ala-Cys- $S_9$
	where $S_8$ is $S_1$ -Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly, (SEQ ID NO:99)
	$S_1$ -Ala-Pro-Ala-Ala-Pro-Ala-Gly, (SEQ ID NO:100)
5	$S_1$ -Pro-Ala-Ala-Pro-Ala-Gly, (SEQ ID NO:101)
	$S_1$ -Ala-Ala-Pro-Ala-Gly, (SEQ ID NO:102)
	$S_1$ -Ala-Pro-Ala-Gly, (SEQ ID NO:103)
	$S_1$ -Pro-Ala-Gly,
	$S_1$ -Ala-Gly,
10	$S_1$ -Gly, or
	$S_1$ ;
	and $S_9$ is Val-Pro-Val- $S_2$ ,
	Val-Pro- $S_2$ ,
	Val- $S_2$ , or
15	$S_2$ .

**Table 21**

	$S_{10}$ -Gly-Aaa-Cys-Ala-Val-Pro-Val- $S_{11}$ , (SEQ ID NO:104)
	where $S_{10}$ is $S_1$ -Asp-Ala-Pro-Ala-Ala-Pro-Ala, (SEQ ID NO:105)
20	$S_1$ -Ala-Pro-Ala-Ala-Pro-Ala, (SEQ ID NO:106)
	$S_1$ -Pro-Ala-Ala-Pro-Ala, (SEQ ID NO:107)
	$S_1$ -Ala-Ala-Pro-Ala, (SEQ ID NO:108)
	$S_1$ -Ala-Pro-Ala,
	$S_1$ -Pro-Ala,
25	$S_1$ -Ala, or
	$S_1$ ;
	and $S_{11}$ is Ala-Val-Pro-Val- $S_2$ , (SEQ ID NO:109)
	Ala-Val-Pro- $S_2$ ,
	Ala-Val- $S_2$ ,

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Ala-S<sub>2</sub>, orS<sub>2</sub>.**Table 22**

5

S<sub>12</sub>-Ala-Gly-Cys-Bbb-S<sub>13</sub>where S<sub>12</sub> isS<sub>1</sub>-Asp-Ala-Pro-Ala-Ala-Bbb, (SEQ ID NO:110)S<sub>1</sub>-Ala-Pro-Ala-Ala-Bbb, (SEQ ID NO:111)

---

S<sub>1</sub>-Pro-Ala-Ala-Bbb,S<sub>1</sub>-Ala-Ala-Bbb,

10

S<sub>1</sub>-Ala-Bbb,S<sub>1</sub>-Bbb, orS<sub>1</sub>;and S<sub>13</sub> isBbb-Ala-Val-Pro-Val-S<sub>2</sub>,Bbb-Ala-Val-Pro-S<sub>2</sub>,

15

Bbb-Ala-Val-S<sub>2</sub>,Bbb-Ala-S<sub>2</sub>,Bbb-S<sub>2</sub>, orS<sub>2</sub>.

20

**Table 23**S<sub>14</sub>-Aaa-Ala-Cys-S<sub>15</sub>where S<sub>14</sub> isS<sub>1</sub>-Asp-Ala-Pro-Ala-Ala, (SEQ ID NO:112)S<sub>1</sub>-Ala-Pro-Ala-Ala, (SEQ ID NO:113)S<sub>1</sub>-Pro-Ala-Ala,

25

S<sub>1</sub>-Ala-Ala,S<sub>1</sub>-Ala, orS<sub>1</sub>;and S<sub>15</sub> isGly-Pro-Ala-Val-Pro-Val-S<sub>2</sub>, (SEQ ID NO:114)Gly-Pro-Ala-Val-Pro-S<sub>2</sub>, (SEQ ID NO:115)

30

Gly-Pro-Ala-Val-S<sub>2</sub>, (SEQ ID NO:116)

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Gly-Pro-Ala-S<sub>2</sub>,Gly-Pro-S<sub>2</sub>,Gly-S<sub>2</sub>, orS<sub>2</sub>.

5

**Table 24**S<sub>16</sub>-Ala-Aaa-Cys-S<sub>17</sub>where S<sub>16</sub> is S<sub>1</sub>-Asp-Ala-Pro-Ala, (SEQ ID NO:117)S<sub>1</sub>-Ala-Pro-Ala,

10

S<sub>1</sub>-Pro-Ala,S<sub>1</sub>-Ala, orS<sub>1</sub>;and S<sub>17</sub> is Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub>, (SEQ ID NO:118)Ala-Gly-Pro-Ala-Val-Pro-S<sub>2</sub>, (SEQ ID NO:119)

15

Ala-Gly-Pro-Ala-Val-S<sub>2</sub>, (SEQ ID NO:120)Ala-Gly-Pro-Ala-S<sub>2</sub>, (SEQ ID NO:121)Ala-Gly-Pro-S<sub>2</sub>,Ala-Gly-S<sub>2</sub>,Ala-S<sub>2</sub>, or

20

S<sub>2</sub>.**Table 25**S<sub>18</sub>-Ala-Ala-Cys-Bbb-S<sub>19</sub>where S<sub>18</sub> is S<sub>1</sub>-Asp-Ala-Bbb,

25

S<sub>1</sub>-Ala-Bbb,S<sub>1</sub>-Bbb, orS<sub>1</sub>;and S<sub>19</sub> is Bbb-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub>, (SEQ ID NO:122)Bbb-Ala-Gly-Pro-Ala-Val-Pro-S<sub>2</sub>, (SEQ ID NO:123)

30

Bbb-Ala-Gly-Pro-Ala-Val-S<sub>2</sub>, (SEQ ID NO:124)

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Bbb-Ala-Gly-Pro-Ala-S<sub>2</sub>, (SEQ ID NO:125)Bbb-Ala-Gly-Pro-S<sub>2</sub>,Bbb-Ala-Gly-S<sub>2</sub>,Bbb-Ala-S<sub>2</sub>,Bbb-S<sub>2</sub>, orS<sub>2</sub>.**Table 26**S<sub>20</sub>-Asp-Ala-Aaa-Ala-Cys- Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>21</sub> (SEQ ID NO:126)where S<sub>20</sub> is S<sub>1</sub>-Asp-Ala,S<sub>1</sub>-Ala, orS<sub>1</sub>;and S<sub>21</sub> is Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub>, (SEQ ID NO:127)Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-S<sub>2</sub>, (SEQ ID NO:128)Ala-Pro-Ala-Gly-Pro-Ala-Val-S<sub>2</sub>, (SEQ ID NO:129)Ala-Pro-Ala-Gly-Pro-Ala-S<sub>2</sub>, (SEQ ID NO:130)Ala-Pro-Ala-Gly-Pro-S<sub>2</sub>, (SEQ ID NO:131)Ala-Pro-Ala-Gly-S<sub>2</sub>, (SEQ ID NO:132)Ala-Pro-Ala-S<sub>2</sub>,Ala-Pro-S<sub>2</sub>,Ala-S<sub>2</sub>, orS<sub>2</sub>.**Table 27**S<sub>22</sub>-Ala-Aaa-Cys-S<sub>23</sub>where S<sub>22</sub> is S<sub>1</sub>-Asp orS<sub>1</sub>;and S<sub>23</sub> is Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub>, (SEQ ID NO:133)Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-S<sub>2</sub>, (SEQ ID NO:134)Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-S<sub>2</sub>, (SEQ ID NO:135)

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Ala-Ala-Pro-Ala-Gly-Pro-Ala-S<sub>2</sub>, (SEQ ID NO:136)Ala-Ala-Pro-Ala-Gly-Pro-S<sub>2</sub>, (SEQ ID NO:137)Ala-Ala-Pro-Ala-Gly-S<sub>2</sub>, (SEQ ID NO:138)Ala-Ala-Pro-Ala-S<sub>2</sub>, (SEQ ID NO:139)5 Ala-Ala-Pro-S<sub>2</sub>,Ala-Ala-S<sub>2</sub>,Ala-S<sub>2</sub>, orS<sub>2</sub>.

10

**Table 28**

S<sub>1</sub>-Asp-Ala-Cys-Bbb-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>24</sub> (SEQ ID NO:140)

where S<sub>24</sub> is Bbb-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val-S<sub>2</sub>, (SEQ ID NO:141)

Bbb-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-S<sub>2</sub>, (SEQ ID NO:142)

15

Bbb-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-S<sub>2</sub>, (SEQ ID NO:143)Bbb-Ala-Ala-Pro-Ala-Gly-Pro-Ala-S<sub>2</sub>, (SEQ ID NO:144)Bbb-Ala-Ala-Pro-Ala-Gly-Pro-S<sub>2</sub>, (SEQ ID NO:145)Bbb-Ala-Ala-Pro-Ala-Gly-S<sub>2</sub>, (SEQ ID NO:146)Bbb-Ala-Ala-Pro-Ala-S<sub>2</sub>, (SEQ ID NO:147)

20

Bbb-Ala-Ala-Pro-S<sub>2</sub>,Bbb-Ala-Ala-S<sub>2</sub>,Bbb-Ala-S<sub>2</sub>,Bbb-S<sub>2</sub>, orS<sub>2</sub>.

25

**Example 4**

A discrete library of peptides was developed based on the known vasopressin ligand Pmp-D-Trp-Ile-Thr-Dap-Cys-Pro-Orn, wherein Pmp is  $\beta$ -mercapto- $\beta$ ,  $\beta$ -cyclopentamethylenepropionyl and Dap is diaminopropionic acid (Chan WY et al.: Discovery and design of novel and selective vasopressin and oxytocin agonists and antagonists: the role of bioassays, *Exp Physiol* 85: Spec

30



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No:7S-18S, 2000). This ligand contains a disulfide bridge between the 1 and 6 residues. The endogenous Cys group was replaced by an Ala, like Cys a relatively small, neutral amino acid. By making such a substitution, the need to protect and the endogenous Cys in the n = 6 position was eliminated. Similarly, cyclohexylacetic acid was substituted in place of the N-terminal  $\beta$ -mercapto- $\beta$ ,  
5  $\beta$ -cyclopentamethylenepropionyl group. By making such substitutions, the need to protect and subsequently deprotect the endogenous -SH groups of the two residues at the 1 and 6 position was eliminated. In addition, some compounds were made with different neutral N-terminal residues, such as cyclohexglycine (Chg), Pmp or D-Chg. The peptides were made as described generally in Example 1, and were complexed with rhenium as described therein. The resulting metallopeptides,  
10 shown in Table 29 below, were then screened for activity.

The screening of metallopeptides for binding to oxytocin receptor was done using cell membranes prepared from rat uterus. A Millipore Multi-Screen System was used for the assay, and was performed in 96-well Millipore filter plates (Durapore, 0.45  $\mu$ m porosity) freshly blocked with 0.5% bovine serum albumin in phosphate buffered saline (PBS). The membrane preparations (10 -  
15 50  $\mu$ g/well) were incubated with 412-800 pM  $^3$ H-oxytocin in HEPES Buffer containing 0.2% bovine serum albumin along with a test compound (1  $\mu$ M final assay concentration) for 2 hours at 4° C. Non-specific binding was determined by addition of  $10^{-6}$  M oxytocin instead of the test compound. After incubation, the membranes were filtered and washed three times with ice-cold PBS. The membranes were air-dried and punched directly into scintillation vials. After addition of the  
20 scintillation cocktail, the vials were capped and gently shaken for 12 hours to dissolve the radioactivity contained in the filters. The vials were then read for tritium counts in a scintillation counter. Specific binding was determined as the radioactivity in wells containing  $^3$ H-oxytocin alone minus the radioactivity in wells containing  $10^{-6}$  M oxytocin. The assay was performed in triplicates. The activity profile for the test compounds were generated by their ability to inhibit specific binding of  
25 the radiotracer to its receptor.

The screening of compounds for vasopressin-1 receptor was performed using cell membranes prepared from rat liver. The assay was essentially performed as described above for the oxytocin receptor assay. In this assay 2-4 nM  $^3$ H-vasopressin-1 antagonist (obtained from Perkin Elemer - NEN Life Sciences) was used as the radiotracer and Arg<sup>8</sup>-vasopressin (1  $\mu$ M final  
30 concentration in the assay) was used to determine non-specific binding. The assay was performed

in triplicates. Activity profile for the test compounds were generated by their ability to inhibit specific binding of the radiotracer to its receptor.

<b>Table 29</b> <b>Peptide Sequences for Re Complexation</b> <b>to Form Metallopeptides and Percent Inhibit</b> <b>of Binding to Oxytocin and Vasopressin Receptors</b>		
Re Complexed Sequence	% Inhibition at 1 $\mu$ M	
	Oxytocin Receptor	Vasopressin-1 Receptor
Caca-D-Trp-Ile-Thr-Dap-Ala-Ala-Orn-Cys-NH <sub>2</sub>	0	23
Caca-D-Trp-Ile-Thr-Dap-Ala-Ala-Cys-Orn-NH <sub>2</sub>	0	0
Caca-D-Trp-Ile-Thr-Dap-Ala-Cys-Ala-Orn-NH <sub>2</sub>	0	0
Caca-D-Trp-Ile-Thr-Dap-Ala-Cys-Pro-Orn-NH <sub>2</sub>	0	0
Caca-D-Trp-Ile-Thr-Dap-Cys-Ala-Pro-Orn-NH <sub>2</sub>	9	22
Caca-D-Trp-Ile-Thr-Cys-Dap-Ala-Pro-Orn-NH <sub>2</sub>	0	0
Caca-D-Trp-Ile-Cys-Thr-Dap-Ala-Pro-Orn-NH <sub>2</sub>	0	52
Pmp-D-Trp-Cys-Ile-Thr-Dap-Ala-Pro-Orn-NH <sub>2</sub>	0	23
Chg-D-Trp-Cys-Ile-Thr-Dap-Ala-Pro-Orn-NH <sub>2</sub>	0	0
D-Chg-D-Trp-Cys-Ile-Thr-Dap-Ala-Pro-Orn-NH <sub>2</sub>	0	8

In the foregoing table the MCD is in italics. "Caca" is cyclohexylacetic acid. It can be seen in **Table 29** that in the sequence Caca-D-Trp-Ile-Thr-Dap-Ala-Ala-Orn-Cys-NH<sub>2</sub> the Pro in the n = 7 position was substituted with an Ala, as is the case in the two succeeding sequences. Thereafter, Pro was utilized. This was done to investigate if the presence of a Pro next to constrained metal-peptide core caused any conformational perturbation. The data on this set of compounds clearly demonstrates that the compounds are selective for the vasopressin receptor and that one of these peptides has the maximal activity. It was remarkable to observe that this peptide is also constrained by metal complexation in the same region as the parent disulfide bridge-constrained peptide. However, in this case, the metal ion induced constraint identified a more specified pair of amino acid residues, D-Trp-Ile, as the main residues structurally organized in a bioactive disposition. In the parent peptide, four amino acid residues, D-Trp-Ile-Thr-Dap, are within the disulfide constraint. The metallopeptide approach, therefore, defined a more precise pharmacophore model.

**Example 5**

A discrete library of peptides was developed based on the known natural oxytocin ligand Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:148). This ligand contains an endogenous Cys at both the 1 and 6 positions. Both endogenous Cys groups were replaced by an Ala, like Cys a relatively small, neutral amino acid. By making such a substitution, the need to protect and subsequently deprotect the endogenous Cys in the 1 and 6 positions was eliminated. The peptides were made as described generally in Example 1, and were complexed with rhenium as described therein. The resulting metallopeptides, shown in Table 30 below, were then screened for activity as described in Example 4.

<b>Table 30</b> <b>Peptide Sequences for Re Complexation</b> <b>to Form Metallopeptides and Percent Inhibit</b> <b>of Binding to Oxytocin and Vasopressin Receptors</b>		
<b>Re Complexed Sequence</b>	<b>% Inhibition</b> <b>at 1 <math>\mu</math>M</b>	
	<b>Oxytocin</b> <b>Receptor</b>	<b>Vasopressin-1</b> <b>Receptor</b>
Ala-Tyr-Ile-Gln-Asn-Ala-Pro-Leu-Gly-Cys-NH <sub>2</sub> (SEQ ID NO:149)	6	0
Ala-Tyr-Ile-Gln-Asn-Ala-Ala-Leu-Gly-Cys-NH <sub>2</sub> (SEQ ID NO:150)	0	0
Ala-Tyr-Ile-Gln-Asn-Ala-Ala-Leu-Cys-Gly-NH <sub>2</sub> (SEQ ID NO:151)	0	0
Ala-Tyr-Ile-Gln-Asn-Ala-Ala-Cys-Leu-Gly-NH <sub>2</sub> (SEQ ID NO:152)	0	1
Ala-Tyr-Ile-Gln-Asn-Ala-Cys-Pro-Leu-Gly-NH <sub>2</sub> (SEQ ID NO:153)	2	0
Ala-Tyr-Ile-Gln-Asn-Ala-Cys-Ala-Leu-Gly-NH <sub>2</sub> (SEQ ID NO:154)	31	0
Ala-Tyr-Ile-Gln-Asn-Cys-Ala-Pro-Leu-Gly-NH <sub>2</sub> (SEQ ID NO:155)	42	0
Ala-Tyr-Ile-Gln-Cys-Asn-Ala-Pro-Leu-Gly-NH <sub>2</sub> (SEQ ID NO:156)	18	0
Ala-Tyr-Ile-Cys-Gln-Asn-Ala-Pro-Leu-Gly-NH <sub>2</sub> (SEQ ID NO:157)	0	0
Ala-Tyr-Cys-Ile-Gln-Asn-Ala-Pro-Leu-Gly-NH <sub>2</sub> (SEQ ID NO:158)	6	1

Here too the MCD is shown in italics, with the endogenous Cys residues in the first and sixth positions replaced with an Ala. The data on this set of compounds demonstrates that the compounds are selective for the oxytocin receptor. The metallopeptide of SEQ ID NO:155 had maximal activity, while the two immediately adjacent metallopeptides, SEQ ID NO:154 and SEQ ID NO:156, were also active. It was again remarkable to observe that the most active metallopeptide

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was also constrained by metal complexation in the same region as the parent disulfide bridge constrained oxytocin. However, in this case, the metal ion induced constraint identified a more specified pair of amino acid residues, Gln-Asn, as the main amino acid residues that were structurally organized in a bioactive disposition. In the parent peptide four amino acid residues are within the disulfide constrain. The metallopeptide approach has therefore identified a more precise pharmacophore model.

#### **Example 6**

A discrete library of peptides were developed based on the known angiotension ligand Sar-Arg-Val-Tyr-Ile-His-Pro-Thr (SEQ ID NO:159), wherein Sar is sarcosine, which served as the parent peptide. (Takei Y. et al., *Gen Comp Endocrinol* 90:214, 1993) The peptides were made as described generally in Example 1, and were complexed with rhenium as described therein. The resulting metallopeptides, shown in **Table 31** below, were then screened for activity as described below.

The screening of compounds for binding to the angiotensin-II receptor was performed using cell membranes obtained from human neuroblastoma cells (KAN-TS). The assay was performed in triplicates, generally as described in Example 4 for oxytocin, except for measurement of receptor bound radioactivity. For angiotensin, a radioiodinated tracer ligand was used (instead of a tritiated ligand), which radioiodinated tracer facilitated direct measurement of bound radioactivity using a gamma counter. A final 1-3 nM concentration of  $^{125}\text{I}$ -Tyr<sup>4</sup>, Sar<sup>1</sup>, Ile<sup>8</sup>-Angiotensin II ligand (obtained from Perkin Elemer – NEN Life Sciences) was used as radiotracer and angiotensin-II (1  $\mu\text{M}$  final assay concentration) was used to measure non-specific binding. After filtration of the incubation medium, followed by washings, drying the filters and punching the filters into test tubes, the filters were counted for radioactivity in a gamma counter. An activity profile for the test compounds was generated by ability to inhibit specific binding of the radiotracer to its receptor.

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<b>Table 31</b> <b>Peptide Sequences for Re Complexation</b> <b>to Form Metallopeptides and Percent Inhibit</b> <b>of Binding to Angiotensin Receptor</b>	
<b>Re Complexed Sequence</b>	<b>% Inhibition at 1 <math>\mu</math>M</b>
Sar-Arg-Val-Tyr-Ile-His-Gly-Cys-Thr (SEQ ID NO:160)	5
Sar-Arg-Val-Tyr-Ile-His-Cys-Pro-Thr (SEQ ID NO:161)	60
Sar-Arg-Val-Tyr-Ile-Cys-His-Pro-Thr (SEQ ID NO:162)	20
Sar-Arg-Val-Tyr-Cys-Ile-His-Pro-Thr (SEQ ID NO:163)	12
Sar-Arg-Val-Cys-Tyr-Ile-His-Pro-Thr (SEQ ID NO:164)	1
Sar-Arg-Cys-Val-Tyr-Ile-His-Pro-Thr (SEQ ID NO:165)	1
Sar-Arg-Val-Tyr-Ile-His-Cys-Gly-Thr (SEQ ID NO:166)	11

Since the Pro was in the next to last position, it was not substituted except in SEQ ID NO:160, where it was substituted with Gly. In SEQ ID NO:166 Gly was substituted for Pro; it can be seen that the percent inhibition with SEQ ID NO:166 is significantly less than in SEQ ID NO:161, which differ only in the substitute of Gly for Pro, thereby demonstrating that the secondary amino group of Pro contributes to binding, and may be employed, in part, to define the pharmacophore of the receptor.

#### **Example 7**

10. A discrete library of peptides were synthesized based on the amyloid beta-protein related peptides of Table 3. The following peptides of Table 32 were synthesized, using an automated peptide synthesis machine, complexed with Re to form a resulting metallopeptide, which was then purified by HPLC.

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<b>Table 32</b> <b>Synthesized Amyloid Beta-Protein Related</b> <b>Peptides For Use in Metallopeptides</b>	
Ac-Leu-Pro-Phe-Phe-Asp-Cys-NH <sub>2</sub> (SEQ ID NO:167)	X
Ac-Leu-Pro-Phe-Phe-Cys-Asp-NH <sub>2</sub> (SEQ ID NO:168)	X
Ac-Leu-Ala-Phe-Phe-Cys-Asp-NH <sub>2</sub> (SEQ ID NO:169)	
Ac-Leu-Ala-Phe-Cys-Phe-Asp-NH <sub>2</sub> (SEQ ID NO:170)	
Ac-Leu-Ala-Cys-Phe-Phe-Asp-NH <sub>2</sub> (SEQ ID NO:171)	
Ac-Leu-Pro-Phe-Phe-Asp-D-Cys-NH <sub>2</sub>	
Ac-Leu-Pro-Phe-Phe-D-Cys-Asp-NH <sub>2</sub>	
Ac-Leu-Ala-Phe-Phe-D-Cys-Asp-NH <sub>2</sub>	
Ac-Leu-Ala-Phe-D-Cys-Phe-Asp-NH <sub>2</sub>	
Ac-Leu-Ala-D-Cys-Phe-Phe-Asp-NH <sub>2</sub>	

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

- 5 Although the invention has been described in detail with particular reference to these preferred embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents. The entire disclosures of all references, applications, patents, and publications cited above are hereby incorporated by reference.

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CLAIMS

What is claimed is:

- 5                   1.       A method of determining a secondary structure binding to a target of interest within a known parent polypeptide that binds to the target of interest, comprising the steps of:
- (a)       providing a known parent polypeptide that binds to a target of interest with a known primary structure, such primary structure consisting of n residues;
- (b)       constructing a first peptide of the formula  $R_1-C-R_2$ ,
- 10                   wherein
- $R_1$  comprises from 2 to n residues, such residues the same as or homologues of residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure;
- C is a residue or mimetic thereof providing both an N and an S for metal ion
- 15       complexation;
- $R_2$  comprises from 0 to n-2 residues, such residues the same as or homologues of residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure, and forming with  $R_1$  a sequence in the same order as in the parent polypeptide primary structure with C either inserted between two adjacent residues corresponding to
- 20       two adjacent residues in such primary structure or substituting for a single residue corresponding to a single residue in such primary structure;
- (c)       complexing the first peptide of the formula  $R_1-C-R_2$  to a metal ion, thereby forming a first  $R_1-C-R_2$  metallopeptide;
- (d)       screening the first  $R_1-C-R_2$  metallopeptide for binding to the target of
- 25       interest;
- (e)       repeating steps (b) through (d) as required, wherein the resulting  $R_1-C-R_2$  metallopeptide differs in at least either  $R_1$  or  $R_2$ ; and
- (f)       selecting the  $R_1-C-R_2$  metallopeptide exhibiting binding to the target of interest, whereby such  $R_1-C-R_2$  metallopeptide comprises the secondary structure binding to the
- 30       target of interest.

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2. The method of claim 1 wherein C is an L- or D-3-mercapto amino acid.

3. The method of claim 2 wherein the L- or D-3-mercapto amino acid is L- or D-  
5 cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homologue of any of the foregoing.

4. The method of claim 1 wherein the metal ion is an ion of V, Mn, Fe, Co, Ni,  
Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po,  
At, Sm, Eu or Gd.

10

5. The method of claim 1 wherein the target of interest is a receptor, antibody,  
toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or  
extracellular protein domain of biological relevance.

15

6. The method of claim 1 wherein screening for binding to the target of interest  
comprises competing a known binding partner for binding to the target of interest with the R<sub>1</sub>-C-R<sub>2</sub>  
metallopeptide.

20

7. The method of claim 6 wherein the known binding partner is the parent  
polypeptide.

8. The method of claim 1 wherein screening for binding to the target of interest  
comprises a functional assay.

25

9. The method of claim 1 wherein the target of interest is a biological receptor  
capable of transmitting a signal, and screening further comprises determining whether the R<sub>1</sub>-C-R<sub>2</sub>  
metallopeptide induces transmission of the signal.

30

10. The method of claim 1 wherein the target of interest is a biological receptor  
capable of transmitting a signal, and screening further comprises determining whether the R<sub>1</sub>-C-R<sub>2</sub>



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metallopeptide inhibits transmission of the signal in the presence of a binding partner to the target of interest known to induce transmission of the signal.

11. The method of claim 1 wherein  $R_1$  and  $R_2$  are each the same as residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure.

12. The method of claim 1 wherein any cysteine residue in  $R_1$  or  $R_2$  is substituted with a homologue not containing a free sulfhydryl group.

13. The method of claim 12 wherein the cysteine is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

14. The method of claim 12 wherein the cysteine is substituted with an S-protected cysteine.

15. The method of claim 12 wherein the cysteine is substituted with a neutral mimetic of an amino acid residue of less than about 150 MW.

16. The method of claim 1 wherein the peptides of the formula  $R_1-C-R_2$  are constructed by a chemical method of peptide synthesis.

17. The method of claim 16 wherein the chemical method of peptide synthesis is solid phase synthesis.

18. The method of claim 16 wherein the chemical method of peptide synthesis is solution phase synthesis.

19. The method of claim 16 wherein C further comprises an orthogonal S-protecting group compatible with the chemical method of peptide synthesis, which orthogonal S-protecting group is cleavable at or prior to metal ion complexation.

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20. The method of claim 1 wherein the peptides of the formula  $R_1-C-R_2$  are constructed by expression in biological systems.

5 21. The method of claim 20 wherein expression in biological systems comprises use of a recombinant vector.

22. The method of claim 1 wherein any proline residues in the two residues immediately adjacent the amino-terminus side of C is substituted.

10

23. The method of claim 22 wherein the proline is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

24. The method of claim 22 wherein the proline is substituted with a neutral  
15 mimetic of an amino acid of less than about 150 MW and providing an N for metal ion complexation.

25. The method of claim 1 wherein n is at least 3.

26. The method of claim 1 wherein the number of residues comprising  $R_1$  and  $R_2$   
20 is less than n.

27. The method of claim 1 wherein the number of residues comprising  $R_1$  and  $R_2$  is equal to n.

28. The method of claim 1 wherein if n is at least 15 the method further  
25 comprises the step of dividing the primary structure into at least three divided primary structures, each such divided primary structure overlapping the primary structure of each adjacent divided primary structure by at least two residues, and thereafter following steps (b) through (f) with respect to each such divided primary structure.

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29. The method of claim 1 wherein the  $R_1$ -C- $R_2$  metallopeptide is stable in solution.

30. The method of claim 1 wherein the  $R_1$ -C- $R_2$  metallopeptide is a stable solid  
5 when not in solution.

31. The method of claim 1 wherein the metal ion is Re or Tc.

32. The method of claim 1 wherein the parent polypeptide is a peptide,  
10 polypeptide or protein.

33. The method of claim 1 wherein the peptide of the formula  $R_1$ -C- $R_2$  further comprises an N-terminus free amino group or acetyl group.

34. The method of claim 1 wherein the peptide of the formula  $R_1$ -C- $R_2$  further comprises a C-terminus free carboxylate or amide group.

35. A method of determining a secondary structure binding to a target of interest within a parent polypeptide with a known primary structure that binds to the target of interest,  
20 comprising the steps of:

(a) providing a parent polypeptide with a known primary structure that binds to a target of interest comprising  $n$  amino acid residues, wherein  $n$  is at least 3;

(b) constructing at least one construct comprised of at least three elements, wherein one element is an  $N_1S_1$  element with an  $\alpha$ -amino group and providing both an N  
25 and an S for complexation to a metal ion, the metal ion to be provided, and at least two elements each comprise an  $\alpha$ -amino group and an  $\alpha$ -carboxyl group and providing an N for complexation to a metal ion, the metal ion to be provided, such at least two elements being the same as or homologous with and in the same order as residues in the parent polypeptide with a known primary structure, the at least three elements being joined by peptide bonds and ordered such that the  $N_1S_1$  element is on

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the carboxyl terminus end of the at least two elements, thereby forming an N<sub>1</sub>S<sub>1</sub> element-containing construct;

(c) complexing the resulting N<sub>1</sub>S<sub>1</sub> element-containing construct to a metal ion, thereby forming a metalloconstruct;

5 (d) screening the metalloconstruct for binding to the target of interest;

(e) repeating steps (b) through (d) as required, with the remaining at least two elements the same as or homologous with and in the same order as a sequence comprising at least one different residue in the parent polypeptide with a known primary structure; and

10 (f) selecting the metalloconstruct exhibiting the highest binding to the target of interest.

36. The method of claim 35 wherein the N<sub>1</sub>S<sub>1</sub> element is the carboxyl terminal end element of the construct.

15

37. The method of claim 35 wherein the N<sub>1</sub>S<sub>1</sub> element is not the carboxyl terminal end element of the construct.

38. The method of claim 35 wherein the N<sub>1</sub>S<sub>1</sub> element-containing construct  
20 comprises at least four elements, said four elements comprising an N<sub>1</sub>S<sub>1</sub> element with an α-amino group and the remaining at least three elements each comprise an α-amino group and an α-carboxyl group, such remaining at least three elements being the same as or homologous with and in the same order as residues in the parent polypeptide with a known primary structure, wherein the N<sub>1</sub>S<sub>1</sub> element is on the carboxyl terminus end of at least two of the at least three elements, the at least four  
25 elements being joined by peptide bonds.

39. The method of claim 35 wherein the N<sub>1</sub>S<sub>1</sub> element is an L- or D-3-mercapto amino acid.

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40. The method of claim 39 wherein the L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine or a homologue of any of the foregoing.

41. The method of claim 35 wherein the metal ion is an ion of V, Mn, Fe, Co, Ni,  
5 Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po,  
At, Sm, Eu or Gd.

42. The method of claim 35 wherein the metal ion is Re or Tc.

10 43. The method of claim 35 wherein the metalloconstruct is stable in solution.

44. The method of claim 35 wherein the metalloconstruct is a stable solid when not in solution.

15 45. The method of claim 35 wherein the target of interest is a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

20 46. The method of claim 35 wherein screening for binding to the target of interest comprises competing a known binding partner for binding to the target of interest with the metalloconstruct.

47. The method of claim 46 wherein the known binding partner is the parent polypeptide.

25 48. The method of claim 35 wherein screening for binding to the target of interest comprises a functional assay.

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49. The method of claim 35 wherein the target of interest is a biological receptor capable of transmitting a signal, and screening further comprises determining whether the metalloconstruct induces transmission of the signal.

5 50. The method of claim 35 wherein the target of interest is a biological receptor capable of transmitting a signal, and screening further comprises determining whether the metalloconstruct inhibits transmission of the signal in the presence of a binding partner to the target of interest known to induce transmission of the signal.

10 51. The method of claim 35 wherein the at least two elements comprise amino acid residues.

52. The method of claim 51 wherein the amino acid residues comprise alanine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine,  
15 asparagine, methionine, proline, glutamine, arginine, serine, threonine, valine, tryptophan or tyrosine.

53. The method of claim 51 wherein the amino acid residues are L-amino acid residues.

20 54. The method of claim 51 wherein the amino acid residues are D-amino acid residues.

55. The method of claim 51 wherein the amino acid residues comprise L-amino acid residues and D-amino acid residues.

25 56. The method of claim 51 wherein the amino acid residues comprise modified protein amino acid residues, non-protein amino acid residues, mimetics of non-protein amino acid residues, mimetics of protein amino acid residues, post-translationally modified amino acid residues, or enzymatically modified amino acid residues.

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57. The method of claim 35 wherein the at least two amino acid residues are each the same as and in the same order as residues in the parent polypeptide with a known primary structure.

5 58. The method of claim 35 wherein any cysteine residue in that portion of the parent polypeptide with a known primary structure that is the same as or homologous with elements other than the N<sub>1</sub>S<sub>1</sub> element with an  $\alpha$ -amino group is substituted with a homologue without a free sulfhydryl group.

10 59. The method of claim 58 wherein the homologue without a free sulfhydryl group for cysteine is a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

60. The method of claim 58 wherein the homologue without a free sulfhydryl group for cysteine is an S-protected cysteine.

15 61. The method of claim 58 wherein the homologue without a free sulfhydryl group for cysteine is a neutral mimetic of an amino acid of less than about 150 MW.

20 62. The method of claim 35 wherein the construct is constructed by a chemical method of peptide synthesis.

63. The method of claim 62 wherein the chemical method of peptide synthesis is solid phase synthesis.

25 64. The method of claim 62 wherein the chemical method of peptide synthesis is solution phase synthesis.

65. The method of claim 62 wherein the N<sub>1</sub>S<sub>1</sub> element with an  $\alpha$ -amino group further comprises an orthogonal S-protecting group bound to the S and compatible with the chemical

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method of peptide synthesis, which orthogonal S-protecting group is cleavable at or prior to metal ion complexation.

5                   66.     The method of claim 35 wherein the constructs are constructed by expression in biological systems.

                  67.     The method of claim 66 wherein expression in biological systems comprises use of a recombinant vector.

10                  68.     The method of claim 35 wherein any proline residue in that portion of the parent polypeptide with a known primary structure that is the same as or homologous with the two elements adjacent the amino-terminus side of the N<sub>1</sub>S<sub>1</sub> element with an  $\alpha$ -amino group is substituted with a homologue providing an N for complexing to a metal ion.

15                  69.     The method of claim 68 wherein the homologue is a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

                  70.     The method of claim 68 wherein the homologue is a neutral mimetic of an amino acid of less than about 150 MW.

20                  71.     The method of claim 35 wherein the number of elements is less than n.

                  72.     The method of claim 35 wherein the number of elements is equal to n.

25                  73.     The method of claim 35 wherein the number of elements is equal to n + 1.

                  74.     The method of claim 35, wherein the parent polypeptide is a peptide, polypeptide or protein.



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75. The method of claim 35 wherein the metalloconstruct further comprises an N-terminus free amino group or acetyl group.

5 76. The method of claim 35 wherein the metalloconstruct further comprises a C-terminus free carboxylate or amide group.

77. A method of determining a metallopeptide that binds to a target of interest, comprising the steps of:

10 (a) selecting a known amino acid sequence with a known primary structure of n residues, where n is at least 3, which known amino acid sequence binds to the target of interest;

(b) designing a library of amino acid sequences by selecting at least two consecutive residues from a stretch of consecutive residues in the known primary structure and inserting a residue providing both an N and S for metal ion complexation on the carboxy terminal end of two of the at least two selected consecutive residues, each such sequence constituting a library member, wherein each library member differs by at least one residue or the location of the insertion of the residue providing both an N and S for metal ion complexation;

15 (c) constructing the library of designed amino acid sequences;  
(d) complexing each library member of designed amino acid sequences to a metal ion, thereby forming a library of metallopeptides;

(e) screening the library of metallopeptides for binding to the target of interest; and

(f) selecting a metallopeptide exhibiting binding to the target of interest.

25 78. The method of claim 77 wherein the known amino acid sequence with a known primary structure of n residues is a peptide, a polypeptide or a protein.

79. The method of claim 77 wherein the library of designed amino acid sequences comprises at least one member wherein the residue providing both an N and S for metal ion complexation is the carboxyl terminal end residue of the amino acid sequence.

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80. The method of claim 77 wherein the library of designed amino acid sequences comprises at least one member wherein the residue providing both an N and S for metal ion complexation is not the carboxyl terminal end residue of the amino acid sequence.

5

81. The method of claim 77 wherein the library of designed amino acid sequences comprises at least one member with at least four residues, wherein the residue providing both an N and S for metal ion complexation is inserted between two adjacent consecutive residues from a stretch of consecutive residues in the known primary structure.

10

82. The method of claim 77 wherein the residue providing both an N and S for metal ion complexation is an L- or D-3-mercapto amino acid.

15

83. The method of claim 82 wherein the L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homologue of any of the foregoing.

20

84. The method of claim 77 wherein the metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or Gd.

85. The method of claim 77 wherein the target of interest is a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

25

86. The method of claim 77 wherein screening for binding to the target of interest comprises competing a known binding partner for binding to the target of interest with members of the library of metallopeptides.

30

87. The method of claim 86 wherein the known binding partner is the known amino acid sequence with a known primary structure of n residues.

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88. The method of claim 86 wherein the known amino acid sequence with a known primary structure of n residues is a protein and the known binding partner is a peptide segment of the protein.

5

89. The method of claim 77 wherein screening for binding to the target of interest comprises a functional assay.

90. The method of claim 77 wherein the target of interest is a biological receptor capable of transmitting a signal, and screening further comprises determining whether the metalloptides induce transmission of the signal.

10

91. The method of claim 77 wherein the target of interest is a biological receptor capable of transmitting a signal, and screening further comprises determining whether the metalloptides inhibit transmission of the signal in the presence of a binding partner to the target of interest known to induce transmission of the signal.

15

92. The method of claim 77 wherein any cysteine residue in the library of amino acid sequences other than the inserted residue providing both an N and S for metal ion complexation is substituted with a homologue not containing a free sulfhydryl group.

20

93. The method of claim 92 wherein the cysteine is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

25

94. The method of claim 92 wherein the cysteine is substituted with an S-protected cysteine.

95. The method of claim 92 wherein the cysteine is substituted with a neutral mimetic of an amino acid residue of less than about 150 MW.

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96. The method of claim 77 wherein the library of amino acid sequences is constructed by a chemical method of peptide synthesis.

5 97. The method of claim 96 wherein the chemical method of peptide synthesis is solid phase synthesis.

98. The method of claim 96 wherein the chemical method of peptide synthesis is solution phase synthesis.

10 99. The method of claim 96 wherein the inserted residue providing both an N and S for metal ion complexation further comprises an orthogonal S-protecting group compatible with the chemical method of peptide synthesis, which orthogonal S-protecting group is cleavable at or prior to metal ion complexation.

15 100. The method of claim 77 wherein the library of amino acid sequences is constructed by expression in biological systems.

101. The method of claim 100 wherein expression in biological systems comprises use of a recombinant vector.

20 102. The method of claim 77 wherein any proline residue in the two residues immediately adjacent the amino-terminus side of the residue providing both an N and S in any library member is substituted with a residue providing an N for metal ion complexation

25 103. The method of claim 102 wherein the proline is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

104. The method of claim 102 wherein the proline is substituted with a neutral mimetic of an amino acid of less than about 150 MW and providing an N for metal ion complexation.

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105. The method of claim 77 wherein if  $n$  is at least 15 the method further comprises the step of dividing the primary structure into at least three divided primary structures, each such divided primary structure overlapping the primary structure of each adjacent divided primary structure by at least two residues, and thereafter following steps (b) through (f) with respect  
5 to each such secondary parent polypeptide.

106. The method of claim 77 wherein the members of the library of metallopeptides are stable in solution.

107. The method of claim 77 wherein the members of the library of metallopeptides are a stable solid when not in solution.

108. The method of claim 77 wherein at least one residue of the selected at least two consecutive residues is a homologue of the corresponding residue in the stretch of consecutive  
15 residues in the known primary structure.

109. The method of claim 77 wherein each designed amino acid sequence further comprises an N-terminus free amino group or acetyl group.

110. The method of claim 77 wherein each designed amino acid sequence further comprises a C-terminus free carboxylate or amide group.

111. A method of determining a metallopeptide that binds to a target of interest, comprising the steps of:

- 25 (a) selecting a known amino acid sequence with a known primary structure of  $n$  residues, where  $n$  is at least 4, which known amino acid sequence binds to the target of interest;
- (b) designing a library of amino acid sequences by selecting at least three consecutive residues from a stretch of consecutive residues in the known primary structure and  
30 substituting a residue providing both an N and S for metal ion complexation for the carboxy terminal

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residue of any consecutive stretch of three of the at least three selected consecutive residues, each such sequence constituting a library member, wherein each library member differs by at least one residue;

- (c) constructing the library of designed amino acid sequences;
- 5 (d) complexing library member of designed amino acid sequences to a metal ion, thereby forming a library of metallopeptides;
- (e) screening the library of metallopeptides for binding to the target of interest; and
- (f) selecting a metallopeptide exhibiting binding to the target of interest.

10

112. The method of claim 111 wherein the known amino acid sequence with a known primary structure of n residues is a peptide, a polypeptide or a protein.

113. The method of claim 111 wherein the library of designed amino acid  
15 sequences comprises at least one member wherein the residue providing both an N and S for metal ion complexation is the carboxyl terminal end residue of the amino acid sequence.

114. The method of claim 111 wherein the library of designed amino acid  
20 sequences comprises at least one member wherein the residue providing both an N and S for metal ion complexation is not the carboxyl terminal end residue of the amino acid sequence.

115. The method of claim 111 wherein the residue providing both an N and S for metal ion complexation is an L- or D-3-mercapto amino acid.

25 116. The method of claim 115 wherein the L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homologue of any of the foregoing.

117. The method of claim 115 wherein the metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po,  
30 At, Sm, Eu or Gd.

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118. The method of claim 111 wherein the target of interest is a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

5

119. The method of claim 111 wherein screening for binding to the target of interest comprises competing a known binding partner for binding to the target of interest with members of the library of metallopeptides.

10

120. The method of claim 119 wherein the known binding partner is the known amino acid sequence with a known primary structure of n residues.

15

121. The method of claim 119 wherein known amino acid sequence with a known primary structure of n residues is a protein and the known binding partner is a peptide segment of the protein.

122. The method of claim 111 wherein screening for binding to the target of interest comprises a functional assay.

20

123. The method of claim 111 wherein the target of interest is a biological receptor capable of transmitting a signal, and screening further comprises determining whether the metallopeptides induce transmission of the signal.

25

124. The method of claim 111 wherein the target of interest is a biological receptor capable of transmitting a signal, and screening further comprises determining whether the metallopeptides inhibit transmission of the signal in the presence of a binding partner to the target of interest known to induce transmission of the signal.

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125. The method of claim 111 wherein any cysteine residue in the library of amino acid sequences other than the inserted residue providing both an N and S for metal ion complexation is substituted with a homologue not containing a free sulfhydryl group.

5 126. The method of claim 125 wherein the cysteine is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

10 127. The method of claim 125 wherein the cysteine is substituted with an S-protected cysteine.

128. The method of claim 125 wherein the cysteine is substituted with a neutral mimetic of an amino acid residue of less than about 150 MW.

15 129. The method of claim 111 wherein the library of amino acid sequences is constructed by a chemical method of peptide synthesis.

130. The method of claim 129 wherein the chemical method of peptide synthesis is solid phase synthesis.

20 131. The method of claim 129 wherein the chemical method of peptide synthesis is solution phase synthesis.

25 132. The method of claim 129 wherein the inserted residue providing both an N and S for metal ion complexation further comprises an orthogonal S-protecting group compatible with the chemical method of peptide synthesis, which orthogonal S-protecting group is cleavable at or prior to metal ion complexation.

30 133. The method of claim 111 wherein the library of amino acid sequences is constructed by expression in biological systems.



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134. The method of claim 133 wherein expression in biological systems comprises use of a recombinant vector.

134. The method of claim 111 wherein any proline residue in the two residues  
5 immediately adjacent the amino-terminus side of the residue providing both an N and S in any library member is substituted with a residue providing an N for metal ion complexation

135. The method of claim 134 wherein the proline is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

10

136. The method of claim 134 wherein the proline is substituted with a neutral mimetic of an amino acid of less than about 150 MW and providing an N for metal ion complexation.

137. The method of claim 111 wherein if n is at least 15 the method further  
15 comprises the step of dividing the primary structure into at least three divided primary structures, each such divided primary structure overlapping the primary structure of each adjacent divided primary structure by at least two residues, and thereafter following steps (b) through (f) with respect to each such divided primary structure.

138. The method of claim 111 wherein the members of the library of  
20 metalloptides are stable in solution.

139. The method of claim 111 wherein the members of the library of metalloptides are a stable solid when not in solution.

25

140. The method of claim 111 wherein at least one residue of the selected at least two consecutive residues is a homologue of the corresponding residue in the stretch of consecutive residues in the known primary structure.

141. The method of claim 111 wherein each designed amino acid sequence further comprises an N-terminus free amino group or acetyl group.

142. The method of claim 111 wherein each designed amino acid sequence  
5 further comprises a C-terminus free carboxylate or amide group.

143. A method of determining a target-specific binding pharmacophore for a target of interest, comprising the steps of:

(a) providing a known amino acid sequence that binds to a target of  
10 interest with a known primary structure, such primary structure consisting of n residues;

(b) constructing a first peptide of the formula  $R_1-C-R_2$ ,

wherein

$R_1$  comprises from 2 to n residues, such residues the same as or homologues of residues in the known amino acid sequence and in the same order as residues in the  
15 known amino acid sequence primary structure;

C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

$R_2$  comprises from 0 to n-2 residues, such residues the same as or homologues of residues in the known amino acid sequence and in the same order as residues in the  
20 known amino acid sequence primary structure, and forming with  $R_1$  a sequence in the same order as in the known amino acid sequence primary structure with C either inserted between two adjacent residues corresponding to two adjacent residues in such primary structure or substituting for a single residue corresponding to a single residue in such primary structure;

(c) complexing the first peptide of the formula  $R_1-C-R_2$  to a metal ion,  
25 thereby forming a first  $R_1-C-R_2$  metallopeptide;

(d) screening the first  $R_1-C-R_2$  metallopeptide for binding to the target of interest;

(e) repeating steps (b) through (d) as required, wherein the resulting  $R_1-C-R_2$  metallopeptide differs in at least either  $R_1$  or  $R_2$ ;

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(f) selecting the  $R_1$ -C- $R_2$  metallopeptide exhibiting binding to the target of interest; and

(g) determining the spatial position of amino acid side chains in and immediately adjacent the metal ion coordination site by building a molecular model based on the coordination geometry of the metal ion, thereby defining a target-specific binding pharmacophore for the target of interest.

144. The method of claim 143 further comprising the step of optimizing binding of the selected  $R_1$ -C- $R_2$  metallopeptide to the target of interest by changing the chirality of one or more of the amino acid residues complexed to the metal ion, or amino acid residues adjacent to the amino acid residues complexed to the metal ion.

145. The method of claim 143 further comprising the step of optimizing binding of the selected  $R_1$ -C- $R_2$  metallopeptide to the target of interest by substituting a natural or synthetic homologue for at least one amino acid residue complexed to the metal ion, or at least one amino acid residue adjacent to the amino acid residues complexed to the metal ion.

146. The method of claim 143 wherein building a molecular model comprises computer-based modeling.

147. The method of claim 143 wherein C is an L- or D-3-mercapto amino acid.

148. The method of claim 147 wherein the L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homologue of any of the foregoing.

149. The method of claim 143 wherein the metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or Gd.

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150. The method of claim 143 wherein the target-specific binding pharmacophore for a target of interest comprises the target-specific binding pharmacophore of a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

5

151. The method of claim 143 wherein screening for binding to the target of interest comprises competing a known binding partner for binding to the target of interest with the R<sub>1</sub>-C-R<sub>2</sub> metallopeptide.

10

152. The method of claim 151 wherein the known binding partner is the known amino acid sequence.

153. The method of claim 143 wherein any cysteine residue in R<sub>1</sub> or R<sub>2</sub> is substituted with a homologue not containing a free sulfhydryl group.

15

154. The method of claim 153 wherein the cysteine is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

20

154. The method of claim 153 wherein the cysteine is substituted with an S-protected cysteine.

155. The method of claim 153 wherein the cysteine is substituted with a neutral mimetic of an amino acid residue of less than about 150 MW.

25

156. The method of claim 153 wherein any proline residue in the two residues immediately adjacent the amino-terminus side of C is substituted.

157. The method of claim 156 wherein the proline is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

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157. The method of claim 156 wherein the proline is substituted with a neutral mimetic of an amino acid of less than about 150 MW and providing an N for metal ion complexation.

158. The method of claim 143 wherein n is at least 3.

5

159. The method of claim 143 wherein if n is at least 15 the method further comprises the step of dividing the primary structure into at least three divided primary structures, each such divided primary structure overlapping the primary structure of each adjacent divided primary structure by at least two residues, and thereafter following steps (b) through (f) with respect to each such divided primary structure.

10

159. The method of claim 143 wherein the known amino acid sequence is a peptide, polypeptide or protein.

15

160. A target-specific binding pharmacophore for a target of interest, the pharmacophore defined by a metallopeptide comprising a residue providing both an N and an S for metal ion complexation and, joined by a peptide bond to the amino-terminus side of such residue, at least two consecutive residues that are the same as or homologues of the same number of consecutive residues of the primary structure of a known sequence of amino acid residues that binds to the target of interest, and a metal ion complexed thereto, wherein the metallopeptide binds to the target of interest, provided that any proline residue in the two residues immediately adjacent the amino-terminus side of the residue providing both an N and an S for metal ion complexation is substituted with a residue providing an N for metal ion complexation, and further provided that any residue with a free sulfhydryl group, other than the residue providing both an N and an S for metal ion complexation, is substituted with a homologue not containing a free sulfhydryl group.

20

25

161. The pharmacophore of claim 160 wherein the residue providing both an N and an S for metal ion complexation is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homologue of any of the foregoing.

30

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162. The pharmacophore of claim 160 wherein the proline is substituted with a neutral mimetic of an amino acid of less than about 150 MW and providing an N for metal ion complexation.

5 163. The pharmacophore of claim 160 wherein any one or more cysteine residues in R<sub>1</sub> or R<sub>2</sub> is substituted with a homologue not containing a free sulfhydryl group.

164. The pharmacophore of claim 160 wherein the residue with a free sulfhydryl group is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

10

165. The pharmacophore of claim 160 wherein the residue with a free sulfhydryl group is substituted with an S-protected cysteine.

15

166. The pharmacophore of claim 160 wherein the residue with a free sulfhydryl group is substituted with a neutral mimetic of an amino acid residue of less than about 150 MW.

167. The pharmacophore of claim 160 further defined by the spatial position of amino acid side chains in and immediately adjacent the metal ion coordination site by a molecular model based on the coordination geometry of the metal ion.

20

168. A library of metallopeptides targeted to a target of interest, each constituent library member comprising:

(a) an amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>,

wherein

25

R<sub>1</sub> comprises from 2 to n residues, such residues the same as or homologues of residues in a known amino acid sequence that binds to the target of interest, such known amino acid sequence having a known primary structure, the residues comprising R<sub>1</sub> in the same order as the residues in the known amino acid sequence primary structure;

30

C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

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$R_2$  comprises from 0 to  $n-2$  residues, such residues the same as or homologues of residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure, and forming with  $R_1$  a sequence in the same order as in the parent polypeptide primary structure with C either inserted between two adjacent residues corresponding to two adjacent residues in such primary structure or substituting for a single residue corresponding to a single residue in such primary structure;

n is the number of residues in the parent amino acid sequence primary structure; and

(b) a metal ion complexed to the amino acid sequence of the formula  $R_1-$

10 C- $R_2$ .

169. The library of claim 168 wherein C is an L- or D-3-mercapto amino acid.

170. The library of claim 169 wherein the L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homologue of any of the foregoing.

171. The library of claim 168 wherein the metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or Gd.

172. The library of claim 168 wherein the target of interest is a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

173. The library of claim 168 wherein  $R_1$  and  $R_2$  are each the same as residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure.

174. The library of claim 168 wherein any cysteine residue in  $R_1$  or  $R_2$  is substituted with a homologue not containing a free sulfhydryl group.

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175. The library of claim 174 wherein the cysteine is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

5 176. The library of claim 174 wherein the cysteine is substituted with an S-protected cysteine.

177. The library of claim 174 wherein the cysteine is substituted with a neutral mimetic of an amino acid residue of less than about 150 MW.

10 178. The library of claim 168 wherein the amino acid sequences of the formula  $R_1$ -C- $R_2$  are constructed by a chemical method of peptide synthesis.

179. The library of claim 178 wherein the chemical method of peptide synthesis is solid phase synthesis.

15

180. The library of claim 178 wherein the chemical method of peptide synthesis is solution phase synthesis.

20 181. The library of claim 178 wherein C further comprises an orthogonal S-protecting group compatible with the chemical method of peptide synthesis, which orthogonal S-protecting group is cleaved at or prior to metal ion complexation.

182. The library of claim 168 wherein any proline residue in the two residues immediately adjacent the amino-terminus side of C is substituted.

25

183. The library of claim 182 wherein the proline is substituted with a glycine, alanine, serine, aminoisobutyric acid or dehydroalanine residue.

30 184. The library of claim 182 wherein the proline is substituted with a neutral mimetic of an amino acid of less than about 150 MW and providing an N for metal ion complexation.



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185. The library of claim 168 wherein the constituent library members are stable in solution.

5 186. The library of claim 168 wherein each constituent library member is a stable solid when not in solution.

187. The library of claim 168 wherein the parent amino acid sequence is a peptide, polypeptide or protein.

10

188. The library of claim 168 wherein each constituent library member further comprises an N-terminus free amino group or acetyl group.

189. The library of claim 168 wherein each constituent library member further  
15 comprises a C-terminus free carboxylate or amide group.

190. A library of metallopeptides targeted to the uPA receptor, each constituent library member comprising:

(a) an amino acid sequence of the formula  $R_1-C-R_2$ ,

20

wherein

$R_1$  comprises from 2 to 11 residues, such residues the same as or homologues of residues in the sequence Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp (SEQ ID NO:2), the residues comprising  $R_1$  in the same order as the residues in the SEQ ID NO:2;

C is a residue or mimetic thereof providing both an N and an S for metal ion  
25 complexation;

$R_2$  comprises from 0 to 9 residues, such residues the same as or homologues of residues in SEQ ID NO:2 and forming with  $R_1$  a sequence in the same order as in SEQ ID NO:2 with C either inserted between two adjacent residues corresponding to two adjacent residues in SEQ ID NO:2 or substituting for a single residue corresponding to a single residue in  
30 SEQ ID NO:2; and

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(b) a metal ion complexed to the amino acid sequence of the formula  $R_1$ -C- $R_2$ .

191. The library of claim 190 comprising the constituent library members Ac-Val-Ser-Cys-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:4), Ac-Val-Ser-Asn-Cys-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:5), Ac-Val-Ser-Asn-Lys-Cys-Tyr-Phe-Ser-Asn-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:6), Ac-Val-Ser-Asn-Lys-Tyr-Cys-Phe-Ser-Asn-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:7), Ac-Val-Ser-Asn-Lys-Tyr-Phe-Cys-Ser-Asn-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:8), Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Cys-Asn-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:9), Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Cys-Ile-His-Trp-NH<sub>2</sub> (SEQ ID NO:10), Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-Cys-His-Trp-NH<sub>2</sub> (SEQ ID NO:11), Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Cys-Trp-NH<sub>2</sub> (SEQ ID NO:3), or Ac-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-Cys-NH<sub>2</sub> (SEQ ID NO:12).

192. A library of metallopeptides targeted to a melanocortin receptor, each constituent library member comprising:

(a) an amino acid sequence of the formula  $R_1$ -C- $R_2$ ,

wherein

$R_1$  comprises from 2 to 6 residues, such residues the same as or homologues of residues in the sequence Nle-Ala-His-D-Phe-Arg-Trp, the residues comprising  $R_1$  in the same order as the residues in the Nle-Ala-His-D-Phe-Arg-Trp;

C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

$R_2$  comprises from 0 to 4 residues, such residues the same as or homologues of residues in Ala-His-D-Phe-Arg-Trp and forming with  $R_1$  a sequence in the same order as in Nle-Ala-His-D-Phe-Arg-Trp with C either inserted between two adjacent residues corresponding to two adjacent residues in Nle-Ala-His-D-Phe-Arg-Trp or substituting for a single residue corresponding to a single residue in Nle-Ala-His-D-Phe-Arg-Trp; and

(b) a metal ion complexed to the amino acid sequence of the formula  $R_1$ -C- $R_2$ .

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193. The library of claim 191 comprising the constituent library members Ac-Nle-Ala-Cys-His-D-Phe-Arg-Trp-NH<sub>2</sub>, Ac-Nle-Ala-His-Cys-D-Phe-Arg-Trp-NH<sub>2</sub>, Ac-Nle-Ala-His-D-Phe-Cys-Arg-Trp-NH<sub>2</sub>, Ac-Nle-Ala-His-D-Phe-Arg-Cys-Trp-NH<sub>2</sub> or Ac-Nle-Ala-His-D-Phe-Arg-Trp-Cys-NH<sub>2</sub>.

5

194. A library of amyloid beta-protein related peptides for treatment of Alzheimer's disease, each constituent library member comprising:

(a) an amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>,

wherein

10

R<sub>1</sub> comprises from 2 to 11 residues, such residues the same as or homologues of residues in the sequence His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val (SEQ ID NO:13), the residues comprising R<sub>1</sub> in the same order as the residues in the SEQ ID NO:13;

C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

15

R<sub>2</sub> comprises from 0 to 9 residues, such residues the same as or homologues of residues in SEQ ID NO:13 and forming with R<sub>1</sub> a sequence in the same order as in SEQ ID NO:13 with C either inserted between two adjacent residues corresponding to two adjacent residues in SEQ ID NO:13 or substituting for a single residue corresponding to a single residue in SEQ ID NO:13; and

20

(b) a metal ion complexed to the amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>.

195. A library of amyloid beta-protein related peptides for treatment of Alzheimer's disease, each constituent library member comprising:

25

(a) an amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>,

wherein

R<sub>1</sub> comprises from 2 to 5 residues, such residues the same as or homologues of residues in the sequence Leu-Ala-Phe-Phe-Asp (SEQ ID NO:14), the residues comprising R<sub>1</sub> in the same order as the residues in the SEQ ID NO:14;

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C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

R<sub>2</sub> comprises from 0 to 3 residues, such residues the same as or homologues of residues in SEQ ID NO:14 and forming with R<sub>1</sub> a sequence in the same order as in  
5 SEQ ID NO:14 with C either inserted between two adjacent residues corresponding to two adjacent residues in SEQ ID NO:14 or substituting for a single residue corresponding to a single residue in SEQ ID NO:14; and

(b) a metal ion complexed to the amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>.

10

196. A library of amyloid beta-protein related peptides for treatment of Alzheimer's disease, each constituent library member comprising:

(a) an amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>,

wherein

15

R<sub>1</sub> comprises from 2 to 5 residues, such residues the same as or homologues of residues in the sequence Leu-Pro-Phe-Phe-Asp (SEQ ID NO:15), the residues comprising R<sub>1</sub> in the same order as the residues in the SEQ ID NO:15;

C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

20

R<sub>2</sub> comprises from 0 to 3 residues, such residues the same as or homologues of residues in SEQ ID NO:15 and forming with R<sub>1</sub> a sequence in the same order as in SEQ ID NO:15 with C either inserted between two adjacent residues corresponding to two adjacent residues in SEQ ID NO:15 or substituting for a single residue corresponding to a single residue in SEQ ID NO:15; and

25

(b) a metal ion complexed to the amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>.

197. A library of peptides for treatment of prion disease, each constituent library member comprising:

30

(a) an amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>,

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wherein

R<sub>1</sub> comprises from 2 to 13 residues, such residues the same as or homologues of residues in the sequence Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val (SEQ ID NO:66), the residues comprising R<sub>1</sub> in the same order as the residues in the SEQ ID NO:66;

5 C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

R<sub>2</sub> comprises from 0 to 11 residues, such residues the same as or homologues of residues in SEQ ID NO:66 and forming with R<sub>1</sub> a sequence in the same order as in SEQ ID NO:66 with C either inserted between two adjacent residues corresponding to two adjacent  
10 residues in SEQ ID NO:66 or substituting for a single residue corresponding to a single residue in SEQ ID NO:66; and

(b) a metal ion complexed to the amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>.

15 198. A library of peptides targeting a vasopressin receptor, each constituent library member comprising:

(a) an amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>,

wherein

R<sub>1</sub> comprises from 2 to 8 residues, such residues the same as or  
20 homologues of residues in the sequence d(CH<sub>2</sub>)<sub>5</sub>-D-Trp-Ile-Thr-Dap-Cys-Pro-Orn, the residues comprising R<sub>1</sub> in the same order as the residues in d(CH<sub>2</sub>)<sub>5</sub>-D-Trp-Ile-Thr-Dap-Cys-Pro-Orn;

C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

R<sub>2</sub> comprises from 0 to 6 residues, such residues the same as or  
25 homologues of residues in d(CH<sub>2</sub>)<sub>5</sub>-D-Trp-Ile-Thr-Dap-Cys-Pro-Orn and forming with R<sub>1</sub> a sequence in the same order as in d(CH<sub>2</sub>)<sub>5</sub>-D-Trp-Ile-Thr-Dap-Cys-Pro-Orn with C either inserted between two adjacent residues corresponding to two adjacent residues in d(CH<sub>2</sub>)<sub>5</sub>-D-Trp-Ile-Thr-Dap-Cys-Pro-Orn or substituting for a single residue corresponding to a single residue in d(CH<sub>2</sub>)<sub>5</sub>-D-Trp-Ile-Thr-Dap-Cys-Pro-Orn; and

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(b) a metal ion complexed to the amino acid sequence of the formula  $R_1$ -C- $R_2$ .

198. The library of claim 198 comprising the constituent library members Caca-D-Trp-Ile-Thr-Dap-Ala-Ala-Orn-Cys-NH<sub>2</sub>, Caca-D-Trp-Ile-Thr-Dap-Ala-Ala-Cys-Orn-NH<sub>2</sub>, Caca-D-Trp-Ile-Thr-Dap-Ala-Cys-Ala-Orn-NH<sub>2</sub>, Caca-D-Trp-Ile-Thr-Dap-Ala-Cys-Pro-Orn-NH<sub>2</sub>, Caca-D-Trp-Ile-Thr-Dap-Cys-Ala-Pro-Orn-NH<sub>2</sub>, Caca-D-Trp-Ile-Thr-Cys-Dap-Ala-Pro-Orn-NH<sub>2</sub>, Caca-D-Trp-Ile-Cys-Thr-Dap-Ala-Pro-Orn-NH<sub>2</sub>, Pmp-D-Trp-Cys-Ile-Thr-Dap-Ala-Pro-Orn-NH<sub>2</sub>, Chg-D-Trp-Cys-Ile-Thr-Dap-Ala-Pro-Orn-NH<sub>2</sub>, or D-Chg-D-Trp-Cys-Ile-Thr-Dap-Ala-Pro-Orn-NH<sub>2</sub>.

200. A library of peptides targeting an oxytocin receptor, each constituent library member comprising:

(a) an amino acid sequence of the formula  $R_1$ -C- $R_2$ ,

wherein

$R_1$  comprises from 2 to 9 residues, such residues the same as or homologues of residues in the sequence Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ ID NO:148), the residues comprising  $R_1$  in the same order as the residues in SEQ ID NO:142;

C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

$R_2$  comprises from 0 to 7 residues, such residues the same as or homologues of residues in SEQ ID NO:142 and forming with  $R_1$  a sequence in the same order as in SEQ ID NO:142 with C either inserted between two adjacent residues corresponding to two adjacent residues SEQ ID NO:142 or substituting for a single residue corresponding to a single residue in SEQ ID NO:142; and

(b) a metal ion complexed to the amino acid sequence of the formula  $R_1$ -C- $R_2$ .

201. The library of claim 200 comprising the constituent library members Ala-Tyr-Ile-Gln-Asn-Ala-Pro-Leu-Gly-Cys-NH<sub>2</sub> (SEQ ID NO:149), Ala-Tyr-Ile-Gln-Asn-Ala-Ala-Leu-Gly-Cys-NH<sub>2</sub> (SEQ ID NO:150), Ala-Tyr-Ile-Gln-Asn-Ala-Ala-Leu-Cys-Gly-NH<sub>2</sub> (SEQ ID NO:151), Ala-Tyr-Ile-

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Gln-Asn-Ala-Ala-Cys-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:152), Ala-Tyr-Ile-Gln-Asn-Ala-Cys-Pro-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:153), Ala-Tyr-Ile-Gln-Asn-Ala-Cys-Ala-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:154), Ala-Tyr-Ile-Gln-Asn-Cys-Ala-Pro-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:155), Ala-Tyr-Ile-Gln-Cys-Asn-Ala-Pro-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:156), Ala-Tyr-Ile-Cys-Gln-Asn-Ala-Pro-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:157) or Ala-Tyr-Cys-Ile-Gln-Asn-Ala-Pro-Leu-Gly-NH<sub>2</sub> (SEQ ID NO:158).

202. A library of peptides targeting an angiotensin receptor, each constituent library member comprising:

(a) an amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>,

10 wherein

R<sub>1</sub> comprises from 2 to 8 residues, such residues the same as or homologues of residues in the sequence Sar-Arg-Val-Tyr-Ile-His-Pro-Thr (SEQ ID NO:159), the residues comprising R<sub>1</sub> in the same order as the residues in SEQ ID NO:159;

15 C is a residue or mimetic thereof providing both an N and an S for metal ion complexation;

R<sub>2</sub> comprises from 0 to 6 residues, such residues the same as or homologues of residues in SEQ ID NO:159 and forming with R<sub>1</sub> a sequence in the same order as in SEQ ID NO:159 with C either inserted between two adjacent residues corresponding to two adjacent residues SEQ ID NO:159 or substituting for a single residue corresponding to a single residue in  
20 SEQ ID NO:159; and

(b) a metal ion complexed to the amino acid sequence of the formula R<sub>1</sub>-C-R<sub>2</sub>.

203. The library of claim 200 comprising the constituent library members Sar-Arg-Val-Tyr-Ile-His-Gly-Cys-Thr (SEQ ID NO:160), Sar-Arg-Val-Tyr-Ile-His-Cys-Pro-Thr (SEQ ID NO:161), Sar-Arg-Val-Tyr-Ile-Cys-His-Pro-Thr (SEQ ID NO:162), Sar-Arg-Val-Tyr-Cys-Ile-His-Pro-Thr (SEQ ID NO:163), Sar-Arg-Val-Cys-Tyr-Ile-His-Pro-Thr (SEQ ID NO:164), Sar-Arg-Cys-Val-Tyr-Ile-His-Pro-Thr (SEQ ID NO:165), or Sar-Arg-Val-Tyr-Ile-His-Cys-Gly-Thr (SEQ ID NO:166).

30

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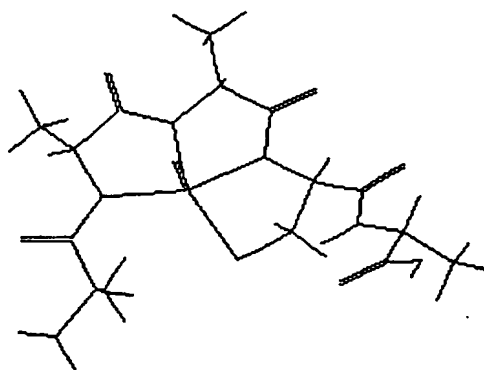


FIG 1 A

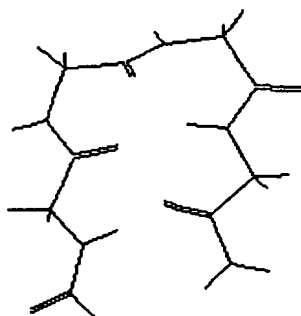


FIG 1 B

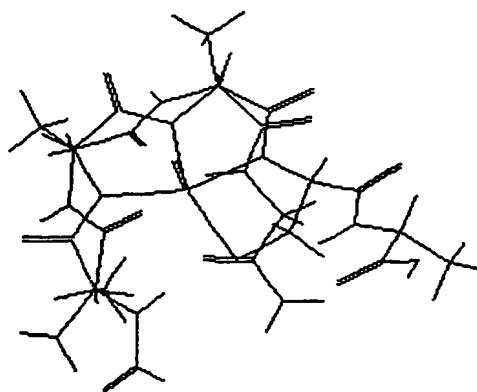


FIG 1 C



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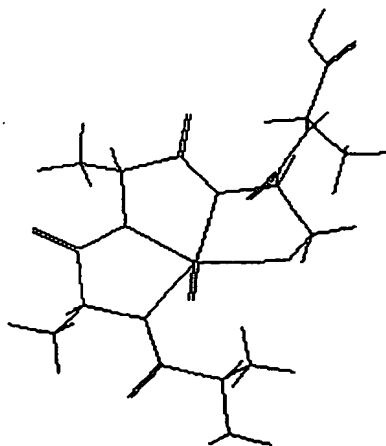


FIG 2 A

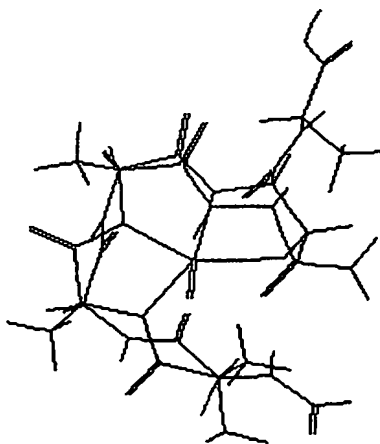


FIG 2 B

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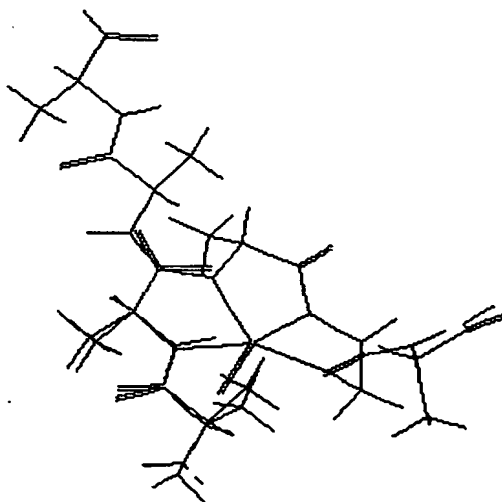


FIG 3 A

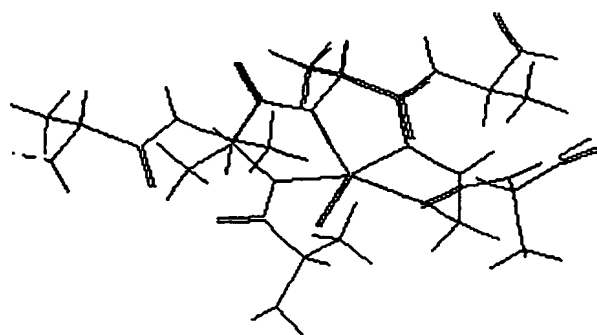


FIG 3 B

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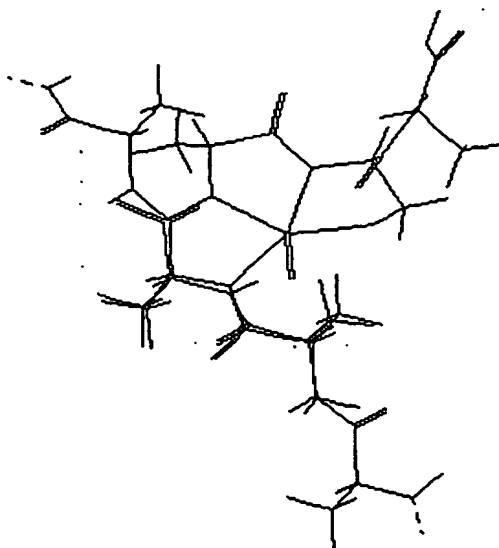


FIG 4 A

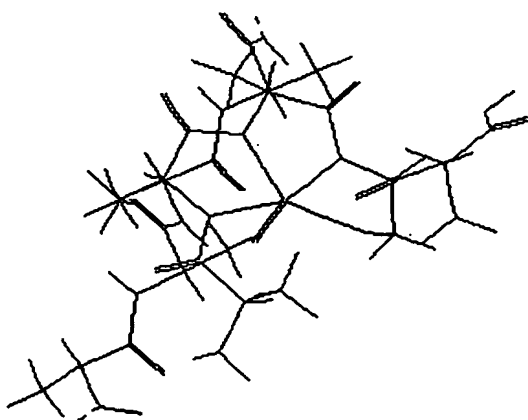


FIG 4 B

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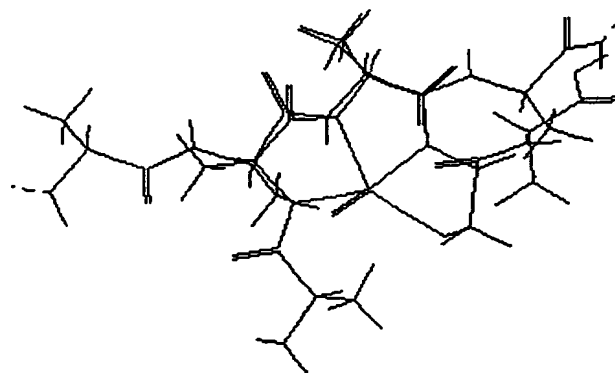


FIG 4 C

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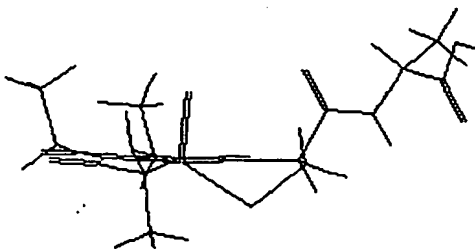


FIG 5

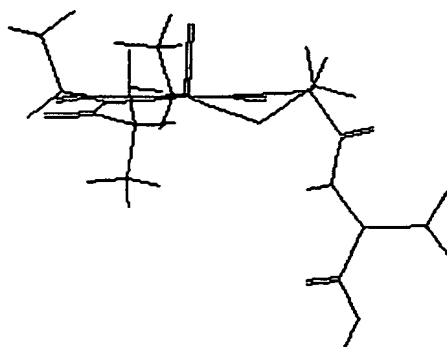


FIG 6

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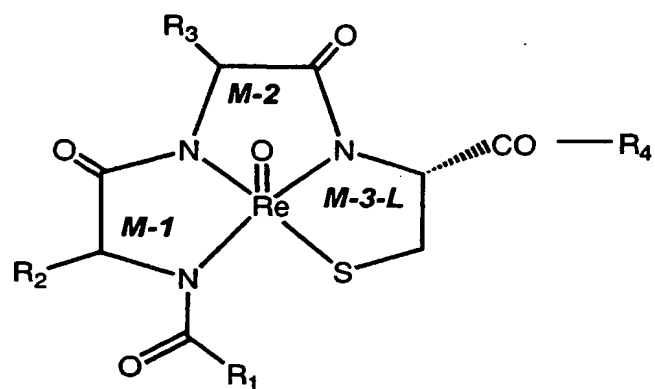


FIG 7 A

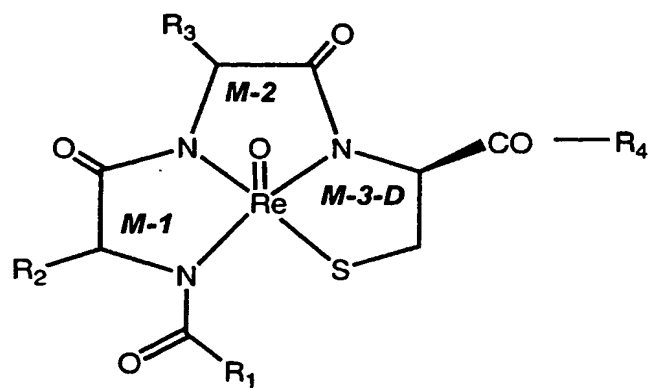


FIG 7 B

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# Phi - Psi Plot

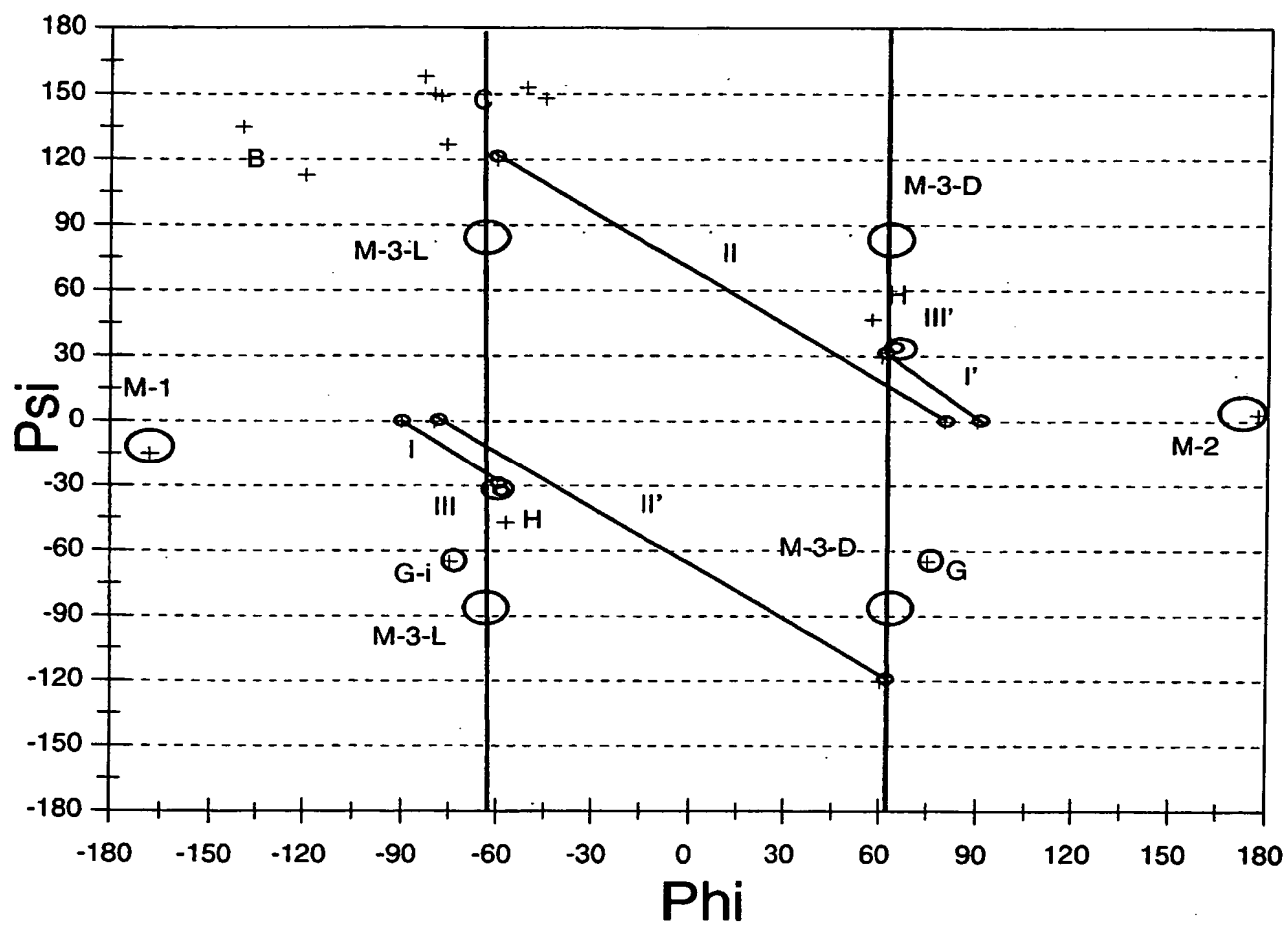


FIG 8

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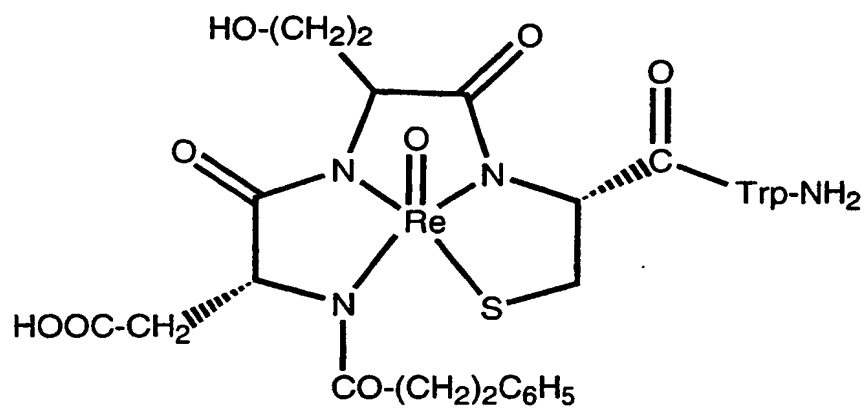


FIG 9 A

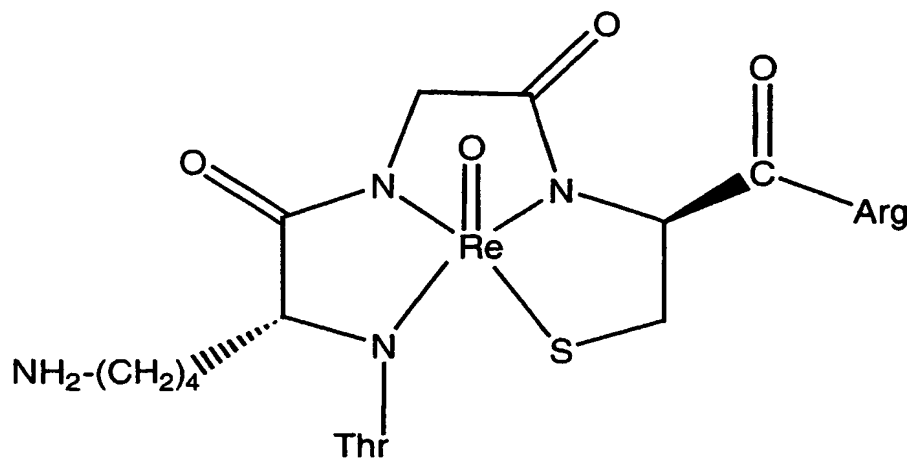


FIG 9 B



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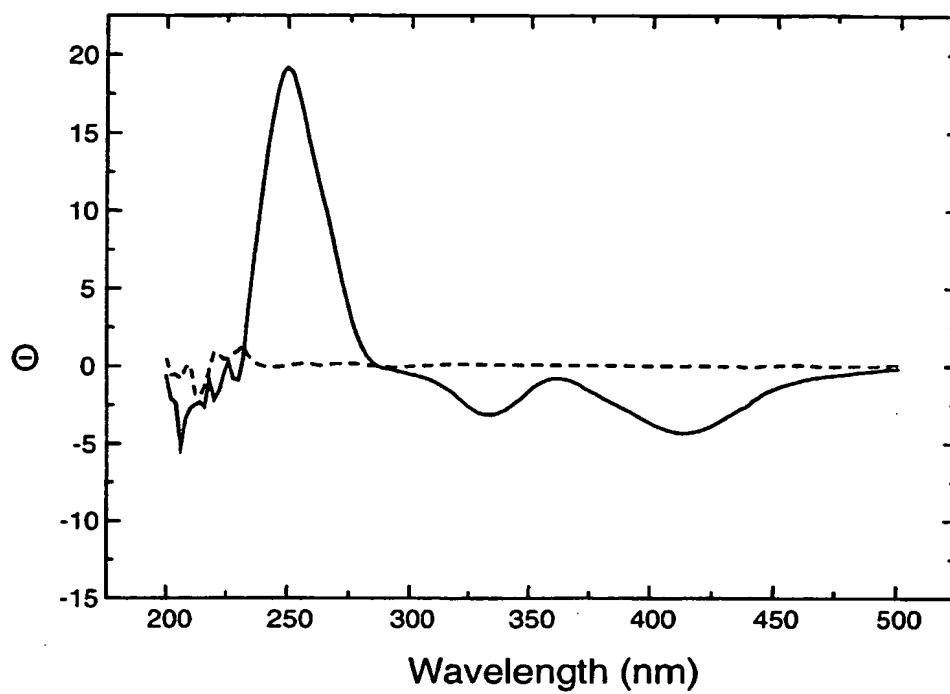


FIG 10 A

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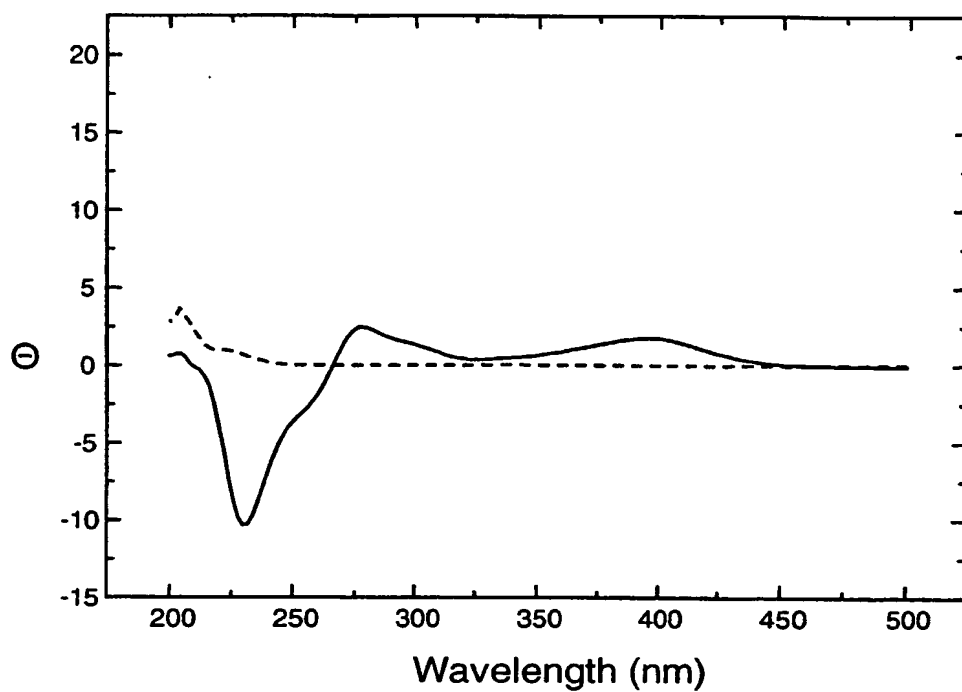


FIG 10 B

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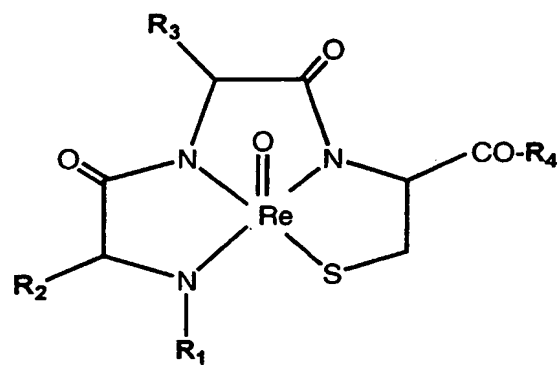


FIG 11

## SEQUENCE LISTING

<110> Palatin Technologies, Inc.  
Sharma, Shubh D.  
Shi, Yi-Qun

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<150> US 60/304,835

<151> 2001-02-13

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&lt;223&gt; Pro, Gly or Ala

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (13)..(13)

&lt;223&gt; Pro, Gly or Ala

&lt;400&gt; 69

Asp Ala Pro Ala Ala Pro Ala Gly Xaa Ala Val Cys Xaa Val  
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&lt;210&gt; 70

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&lt;223&gt; Gly or Ala

&lt;400&gt; 70

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&lt;210&gt; 71

&lt;211&gt; 14

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&lt;223&gt; Gly or Ala

&lt;400&gt; 71

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<400> 72

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<400> 73

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Pro Ala Ala Pro Ala Gly Pro Ala Val  
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Ala Ala Pro Ala Gly Pro Ala Val  
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Ala Pro Ala Gly Pro Ala Val  
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Pro Ala Gly Pro Ala Val  
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Gly Pro Ala Val  
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Ala Pro Ala Ala Pro Ala Gly Pro Ala  
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Ala Gly Pro Ala

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Ala Ala Pro Ala Gly Xaa  
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Ala Pro Ala Ala Pro Ala Gly  
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Pro Ala Ala Pro Ala Gly  
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Ala Ala Pro Ala Gly  
1 5

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<400> 103

Ala Pro Ala Gly  
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Gly Xaa Cys Ala Val Pro Val  
1 5

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Asp Ala Pro Ala Ala Pro Ala  
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Ala Pro Ala Ala Pro Ala  
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<400> 107

Pro Ala Ala Pro Ala  
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Ala Ala Pro Ala  
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Ala Val Pro Val  
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Asp Ala Pro Ala Ala Xaa

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Gly Pro Ala Val  
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Ala Pro Ala Gly  
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Ala Ala Pro Ala Gly Pro Ala Val  
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Ala Ala Pro Ala Gly Pro  
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Ala Ala Pro Ala Gly  
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Xaa Ala Ala Pro Ala Gly Pro Ala Val Pro Val  
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Xaa Ala Ala Pro Ala Gly  
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Xaa Ala Ala Pro Ala  
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Cys Tyr Ile Gln Asn Cys Pro Leu Gly  
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Ala Tyr Ile Gln Asn Ala Ala Leu Gly Cys  
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Ala Tyr Ile Gln Asn Ala Ala Leu Cys Gly  
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Ala Tyr Ile Gln Asn Ala Cys Ala Leu Gly  
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Ala Tyr Ile Gln Asn Cys Ala Pro Leu Gly  
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Ala Tyr Ile Gln Cys Asn Ala Pro Leu Gly  
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